

Summary

This thesis addresses the effects of dietary interventions with lycopene and isoflavone supplementation on the circulating insulin-like growth factor (IGF) system, and also describes molecular studies in which the role of the IGF-system in breast and colorectal carcinogenesis is investigated. The IGF-system is primarily involved in the regulation of prenatal and postnatal growth, and IGF-system components are expressed in most tissues. However, the liver is the principal source for the two ligands IGF-I and IGF-II in the blood circulation. About 90% of IGF-I in the circulation is bound to both IGF binding protein -3 (IGFBP-3) and acid labile subunit. This complex is too large to pass the capillary epithelium, resulting in an increased half-life of IGF-I. Free IGF-I (<1%) and IGF-I bound to IGFBP-1 or IGFBP-2 can be transported out of the bloodstream to specific target tissues. IGFBPs are degradable by proteases, rendering IGF-I free to interact with the IGF-I receptor (IGF-IR). Binding of IGF-I or IGF-II to the IGF-IR results in receptor phosphorylation, activation of downstream targets, and stimulation of proliferation and inhibition of apoptosis.

Epidemiological studies indicate that high circulating IGF-I concentrations are associated with increased risk of cancer, in particular risk of premenopausal breast cancer and colorectal cancer (**Chapters 1 and 2**). High circulating IGF-II concentrations have also been associated with increased colorectal cancer risk. Qualitative and semi-quantitative studies have frequently shown overexpression of the IGF-IR in breast tumor and colorectal tumor tissues. However, results from quantitative studies are scarce and inconsistent. Circulating concentrations of IGF-I are determined by genetic factors as well as dietary and lifestyle factors. *In vitro* and *in vivo* studies and epidemiological studies have shown that the dietary factors lycopene and isoflavones may decrease circulating IGF-I concentrations and increase circulating IGFBP-3 concentrations. Lycopene is a carotenoid primarily present in tomatoes and tomato products. Isoflavones have structural and functional similarities to estrogens, are mainly present in soy foods, but can also be derived from red clover. The effects of lycopene and isoflavones on the IGF-system in humans are tested most optimally in intervention trials using dietary supplements.

The main aim of the studies described in this thesis was to investigate the effects of lycopene and isoflavone supplementation on circulating IGF-I and other IGF-system components in premenopausal women at increased breast cancer risk, and in men and women at increased colorectal cancer risk. Additionally, we examined whether differences exist in expression levels of IGF-system components between normal breast and breast tumor tissues, and we tried to elucidate the relation between levels of IGF-system components in normal colorectal tissue and serum.

Cross-sectional study

We first investigated whether habitual dietary intake of lycopene and tomatoes, phytoestrogens (including isoflavones) and related foods, total energy, protein, and alcohol was associated with plasma levels of IGF-I and IGFBP-1, IGFBP-2, and IGFBP-3 (**Chapter 3**). Therefore, a cross-sectional study was conducted in 224 premenopausal and

162 postmenopausal healthy Dutch women, aged 49-69 years, participating in the Prospect-EPIC study in the Netherlands. Diet was assessed using a food frequency questionnaire. In this study, no independent associations of dietary factors with IGF-I or IGFBP-3 concentrations were observed. Among the lycopene and isoflavone containing foods investigated, only an association between increased intake of soy products and higher plasma IGFBP-2 concentrations in premenopausal women was found ($p = 0.04$). The habitual dietary intake of lycopene and isoflavones in this study population was low (median intake ~3 mg/day and 0.15 mg/day, respectively). Apparently, this low intake was not associated with circulating total IGF-I and IGFBP-1, IGFBP-2, and IGFBP-3 concentrations.

Dietary intervention studies

To accurately examine whether higher intake of lycopene and isoflavones affects circulating IGF-system components, we conducted randomized, placebo-controlled, double-blind cross-over studies with tomato-derived lycopene (30 mg/day) and red clover-derived isoflavone (84 mg/day) supplementation. The total duration of the studies was approximately 6 months, consisting of two 2-month intervention periods separated by a 2-month washout period. Most of the men and women at increased colorectal cancer risk underwent a colonoscopy at the end of the first intervention period. The main parameter of interest was the relative cross-over difference in serum total IGF-I concentrations (i.e., concentration after intervention minus concentration after placebo treatment, expressed as percentage change relative to the concentration after placebo treatment). Relative cross-over differences in serum concentrations of free IGF-I, IGF-II, IGFBP-1, IGFBP-2, and IGFBP-3 were secondary endpoints.

Lycopene and IGF

In **Chapter 4**, we evaluated the effect of lycopene supplementation in premenopausal women with (1) a history of breast cancer ($n = 24$), or (2) a high familial breast cancer risk ($n = 36$), ≤ 50 years of age. Lycopene supplementation did neither significantly alter serum total IGF-I nor any of the other IGF-system components in the two study populations combined. However, statistically significantly discordant results were observed between the two study populations (i.e., $p < 0.05$ for total IGF-I, free IGF-I and IGFBP-3). Total IGF-I and IGFBP-3 were increased in the breast cancer survivor population (mean relative difference between serum total IGF-I concentrations after lycopene supplementation and after placebo, 7.0%, 95%CI -0.2% to 14.3%; IGFBP-3, 3.3%, 95%CI 0.7% to 6.0%), and free IGF-I was decreased in women with a family history of breast cancer (-7.6%, 95%CI -14.6% to -0.6%). No changes were observed in circulating concentrations of IGFBP-1 and IGFBP-2. These results suggest an IGF-I lowering effect of lycopene only in healthy women with a family history of breast cancer, not in breast cancer survivors. This may be due to interference of genetic background or disease status with lycopene effects on the IGF-system. As the IGF-system is known to interact with sex steroid hormone pathways, any

effects of dietary factors on the IGF-system may also differ by gender and menopausal status. However, these results may also be due to chance.

We also investigated the effect of lycopene supplementation in a similar study in 40 men (aged 40-75 years) and 31 postmenopausal women (aged 50-75 years) with a family history of colorectal cancer and/or a personal history of colorectal adenomas (**Chapter 5**). In both men and women, lycopene supplementation did not significantly affect serum total IGF-I concentrations. However, lycopene supplementation significantly increased serum IGFBP-1 concentrations in women (median relative difference 21.7%, $p = 0.01$). Serum IGFBP-2 concentrations were increased in both men and women after lycopene supplementation, but to a lesser extent than IGFBP-1 (mean relative difference 8.2%, 95%CI 0.7% to 15.6%; and 7.8%, 95%CI -5.0% to 20.6%, respectively). IGF-II and IGFBP-3 concentrations were not markedly altered, and free IGF-I concentrations were not measured. Although lycopene supplementation did not influence serum total IGF-I concentrations in men and women at increased colorectal cancer risk, our results indicate that it may decrease IGF-I bioavailability by increasing IGFBP-1 and IGFBP-2 concentrations. However, we were the first to investigate lycopene effects on circulating concentrations of IGFBP-1 and IGFBP-2, and results need to be confirmed in larger randomized intervention studies.

Isoflavones and IGF

The effect of isoflavone supplementation on circulating IGF-system components was investigated in men (aged 40-75 years) and postmenopausal women (aged 50-75 years) with a family history of colorectal cancer and/or a personal history of colorectal adenomas. In 37 men, isoflavone supplementation did not significantly affect serum total IGF-I concentrations (mean relative difference -1.3%, 95%CI -8.6% to 6.0%), or any of the other IGF-system components (**Chapter 6**). Previous studies have shown that about 30-50% of individuals are able to convert daidzein, one of the main isoflavone metabolites, to the more potent estrogenic metabolite equol. Interestingly, in our study higher serum concentrations of equol were associated with decreases in serum IGF-I concentrations after isoflavone supplementation ($r = -0.49$, $p = 0.002$). In conclusion, isoflavone supplementation did not affect circulating concentrations IGF-system components in men at increased colorectal cancer risk. However, to our knowledge, this is the first study that suggests isoflavones might have an IGF-I lowering effect in equol producers only. This underlines the importance of taking into account equol status in future isoflavone intervention studies.

Similarly, isoflavone supplementation did not significantly affect serum concentrations of total IGF-I in 34 postmenopausal women (mean relative difference -2.0%, 95%CI -8.0% to 3.9%) (**Chapter 7**). Neither IGF-II nor IGFBPs were significantly altered after isoflavone supplementation. Moreover, we observed no differences in mRNA expression levels of IGF-system components in normal colorectal tissue biopsies between women on isoflavones and women on placebo. These results suggest that the increased serum IGF-I concentrations observed in previous studies investigating soy food or soy protein

supplementation are most likely due to soy protein itself, and not to isoflavones.

Molecular studies

Breast carcinogenesis

Protein and mRNA expression of IGF-system components in human breast tissue have typically been studied using qualitative or semi-quantitative techniques. Results with respect to mRNA expression in normal breast and breast tumor tissue are inconsistent and inconclusive, and quantitative data on mRNA expression in different types of human breast tissue are lacking. To investigate the plausible causative link between susceptibility (i.e., high serum IGF-I levels and cancer susceptibility) and tumor induction and promotion (i.e., tissue expression and subsequent cancer risk), quantitative data on mRNA expression levels of IGF-system components in breast tissue are essential. In **Chapter 8**, we quantitatively assessed mRNA expression of IGF-I, IGF-II, and their receptors (IGF-IR and IGF-IIR) in breast tissue samples ($n = 83$) from 72 women by real-time RT-PCR. We evaluated whether mRNA expression levels differ in both normal and tumor breast tissue of women with and without a family history of breast cancer. We found a large variation in mRNA levels. Expression of each gene was significantly higher in normal tissue than in tumor tissue (median for normal and tumor tissue, respectively (arbitrary units); IGF-I: 25.2 and 1.4; IGF-II: 5.9 and 0.6; IGF-IR: 0.18 and 0.07; IGF-IIR: 1.8 and 0.9; $p < 0.0001$). Interestingly, in tumor tissue from patients with a strong family history of breast cancer, expression of both receptors was higher than in tumor tissue from sporadic patients (IGF-IR: 0.13 and 0.05, $p = 0.04$; IGF-IIR: 1.1 and 0.8, $p = 0.04$). For cancer-free controls, expression of IGF-II and IGF-IIR in normal breast tissue was also higher in women with a family history of breast cancer than in women without such a family history (IGF-II: 7.2 and 1.5, $p = 0.02$; IGF-IIR: 2.6 and 1.5, $p = 0.09$). Our study quantitatively shows that mRNA expression levels of IGF-system components in the breast are generally higher in normal tissue compared with tumor tissue, and higher in tissue from women with a family history of breast cancer than in tissue from women without such a family history. A basis has therefore been created for studies aimed at understanding IGF as a breast cancer risk factor, the relationship between IGF-systems in serum and tissues, and effects of lifestyle factors on the IGF-system.

Colorectal carcinogenesis

In human colorectal tumors, studies using qualitative or semi-quantitative techniques have shown that the IGF-IR and particularly IGF-II are frequently overexpressed compared with normal colorectal tissue. At present, no quantitative data are available on mRNA expression levels of IGF-system components in different locations of the colon. Moreover, it is unknown whether circulating IGF-I and IGF-II proteins directly affect colorectal tumor growth in humans through IGF-IR binding and activation, whether they influence local tissue expression of IGF-system components (e.g. upregulation of IGF-I, IGF-II, or IGF-IR), or whether they are reflective of tissue IGF-system component expression and thereby act

as a biomarker of tissue IGF-system component bioactivity. To investigate this in more depth, biopsies from macroscopically normal mucosa at four locations in the colorectum (ascending, transverse, and sigmoid colon, rectum) and a fasting serum sample were obtained from 48 asymptomatic patients at increased colorectal cancer risk (**Chapter 9**). We quantitatively evaluated mRNA expression levels of IGF-I, IGF-II, IGF-IR, IGF-IIR, and IGFBP-3 by real-time RT-PCR. Expression of IGF-IR protein in the ascending colon and rectum tissue specimens was assessed semi-quantitatively by immunohistochemistry. Additionally, we studied the relationship of tissue mRNA and protein expression with serum IGF-I and IGF-II concentrations. With the exception of IGF-IIR, mRNA levels of all the IGF-system components investigated, as well as IGF-IR protein expression, were significantly higher in the rectum compared with the ascending colon ($p \leq 0.001$). Circulating IGF-I and IGF-II concentrations did not correlate with any of the parameters studied in colorectal tissues. Our results indicate that in humans IGF-system components are differentially expressed in the colorectum. Moreover, our findings suggest that local and circulating components of the IGF-system are differentially regulated. Our data underline the importance of taking into account the colorectal location when investigating dietary or pharmacological effects on colorectal tissue mRNA expression of IGF-system components.

Conclusions

In **Chapter 10** the findings of our studies are summarized, discussed, and integrated. In our randomized controlled cross-over studies, lycopene did not decrease circulating IGF-I concentrations in women at increased premenopausal breast cancer risk or in men and women at increased colorectal cancer risk. Isoflavones did not decrease circulating IGF-I concentrations in men and women at increased colorectal cancer risk either. These results are in line with results from previous studies. Interestingly, lycopene increased IGFBP-1 concentrations in women and IGFBP-2 concentrations in men and in women at increased colorectal cancer risk, thereby possibly decreasing bioavailable IGF-I. Additionally, our results suggest that isoflavones may decrease circulating IGF-I concentrations in equol producers only, which constitute about 30-50% of the Western population. Both findings need further investigation in future randomized controlled trials. The results of our studies and other evidence published to date do not provide support for health claims for lycopene and isoflavones in lowering circulating IGF-I concentrations in women at increased risk of premenopausal breast cancer and men and women at increased risk of colorectal cancer. Although both circulating and local tissue IGF-system components have been related to breast and colorectal carcinogenesis, we did not find an association between colorectal tissue and circulating levels of these components. Whether circulating IGF-I is causally related to cancer risk in humans needs further investigation.

