NUTRITION AND CANCER, *58*(1), 35–42
Copyright © 2007, Lawrence Erlbaum Associates, Inc.

Relationship of Dietary Protein and Soy Isoflavones to Serum IGF-1 and IGF Binding Proteins in the Prostate Cancer Lifestyle Trial

Antonella Dewell, Gerdi Weidner, Michael D. Sumner, R. James Barnard, Ruth O. Marlin, Jennifer J. Daubenmier, Christine Chi, Peter R. Carroll, and Dean Ornish

Downloaded by [University of California San Francisco] at 09:41 13 October 2011

Abstract: *High levels of insulin-like growth factor 1 (IGF-1) are associated with increased risk of prostate cancer, whereas increased levels of some of its binding proteins (IGFBPs) seem to be protective. High intakes of dietary protein, especially animal and soy protein, appear to increase IGF-1. However, soy isoflavones have demonstrated anti-proliferative and apoptotic effects both in vitro and in vivo. We evaluated dietary intakes of total protein and soy isoflavones in relation to the IGF axis in prostate cancer patients making comprehensive lifestyle changes including a very low-fat vegan diet supplemented with soy protein (58 g/day). After one year, intervention group patients reported significantly higher intakes of dietary protein and soy isoflavones compared to usual-care controls (*$P < 0.001$*). IGF-1 increased significantly in both groups, whereas IGFBP-1 rose in the experimental group only (*$P < 0.01$*). Increases in vegetable protein over one year were associated with increases in IGFBP-1 among intervention group patients (*$P < 0.05$*). These results suggest that dietary protein and soy isoflavones, in the context of comprehensive lifestyle changes, may not significantly alter IGF-1. However, given the recent literature indicating that high intake of protein rich in essential amino acids (animal or soy protein) may increase IGF-1, it may be prudent for men with early stage prostate cancer not to exceed dietary protein recommendations.*

Introduction

Insulin-like growth factor 1 (IGF-1), a hormone that plays an important role in normal growth and development, has also been shown to promote tumor growth and inhibit apoptosis (1,2). A growing body of epidemiological literature suggests that individuals with high IGF-1 levels are at increased risk of various types of cancer, including prostate cancer (3), and especially advanced-stage prostate cancer (4). Conversely, there is some evidence that two of the main IGF-1 binding proteins (IGFBPs), IGFBP-1 and IGFBP-3, may be protective (4–8). In fact, individuals with both the highest levels of IGF-1 and the lowest levels of IGFBP-3 may be those at highest risk (4).

The major determinants of the IGF-1 axis include both genetic and lifestyle factors (e.g., diet and exercise), and age (1,2). Several observational and human feeding studies have identified protein as the most important dietary factor influencing circulating IGF-1 (9–14). Specifically, high intakes of protein rich in essential amino acids (animal or soy protein) are thought to be responsible for the observed increases in IGF-1 (9,12,15). In addition, IGF-1 levels are known to decrease with interventions including a very low-fat diet and/or exercise (5,6). IGF-1 levels are known to increase from birth to puberty and to decline with age afterwards. These changes are regulated by growth hormone. Another important determinant of circulating IGF-1 is insulin, which stimulates its production (16).

Recent research investigating the dietary determinants of IGF-1 has focused on soy protein. The use of soy in Asian diets is theorized to be one of the reasons for the low incidence and mortality rates from prostate cancer in Asian men, which are rapidly increasing with the Westernization of their traditional diet (17). This hypothesis is supported by findings from both laboratory and animal studies, indicating an anti-proliferative and apoptotic effect in cancer cells of isoflavones, the phytoestrogens present in soy (18–21). However, epidemiological and clinical investigations on the role of soy protein in relation to IGF-I suggest that soy protein may alter the IGF axis toward an increase in IGF-1 and a reduction in IGFBP-3 (22–26).

We present a post-hoc analysis of the relationship between dietary intakes of total protein and soy isoflavones to IGF-1, its main binding proteins, IGFBP-1, IGFBP-2, and IGFBP-3, and the IGF-1: IGFBP-3 molar ratio (an estimate of free, i.e., available, IGF-1) in the Prostate Cancer Lifestyle Trial (PCLT). In this randomized, controlled trial,

Antonella Dewell, Gerdi Weidner, Michael D. Sumner, Ruth O. Marlin, Christine Chi, and Dean Ornish are affiliated with Preventive Medicine Research Institute, Sausalito, California. James Barnard is affiliated with University of California, Los Angeles, California. Jennifer J. Daubenmier and Peter R. Carroll are affiliated with University of California, San Francisco, California.

intervention group participants enrolled in a multicomponent lifestyle intervention including a very low-fat vegan diet supplemented with soy protein, exercise, stress management, and group support, and were compared to a usual-care control group (27–29). Specifically, our focus is to evaluate whether high dietary intakes of protein and soy isoflavones (as a measure of soy protein intake, due to the nutrition analysis software's inability to calculate soy protein) are associated with increases in serum IGF-1. Additionally, we investigate associations of dietary protein, soy isoflavones, and exercise, with prostate cancer markers (i.e., prostate specific antigen (PSA), serum-stimulated LNCaP cell growth and apoptosis, and testosterone) and with fasting insulin.

Subjects and Methods

Subjects

The subjects were 93 men participating in the PCLT, a randomized controlled study investigating whether comprehensive diet and lifestyle changes may affect the progression of prostate cancer (27). Participants included men with prostate cancer undergoing active surveillance who were randomized to a lifestyle intervention ($n = 44$) or usual-care control group ($n = 49$). The University of California San Francisco Committee on Human Research Institutional Review Board approved this study. The main findings have been previously reported (27–29). Briefly, patients making intensive lifestyle changes had significantly lower PSA levels and in vitro serum-stimulated LNCaP cell growth compared to patients in the control group (27).

Design

The randomization process, selection criteria, recruitment procedure, intervention, and assessment protocol have been described elsewhere(27,28,30). Briefly, participants in the intervention group were asked to follow an intensive lifestyle program including a very low-fat vegan diet (approximately 10% energy from fat), moderate aerobic exercise (e.g., walking 30 min 6 days weekly), stress management (yoga, breathing, meditation, imagery, and progressive relaxation for a total of 60 min daily), and social group support (1 h weekly). Participants were instructed to consume one to three servings of a soy product and a 58-g serving of a fortified soy protein powdered beverage (*SUPRO® SOY*, The Solae Company, St. Louis, MO, formerly DuPont Technologies), providing 40 g of soy protein and 80 mg of isoflavones (aglycone units) daily. Control group participants were under medical treatment by their personal physician.

Dietary Assessment

Baseline and one-yr nutrient intake data were collected using 3-day food diaries. Dietary intake data were analyzed using Nutrition Data System for Research (NDS-R) software versions 4.01_29 and 4.02_30 (Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN). Final calculations were completed using NDS-R version 2005. The NDS-R time-related database updates analytic data while maintaining nutrient profiles true to the version used for data collection. Isoflavone values specific for the soy protein supplement used in the intervention were provided by the manufacturer and substituted for the isoflavone value estimated by NDS-R.

Serum Analyses

Serum IGF-I, IGFBP-1, IGFBP-2, IGFBP-3, and insulin were measured in duplicate using commercial ELISA kits (Diagnostic Systems Laboratories, Webster, TX). All assays were performed in a blinded manner, and control samples provided by the manufacturer were included in each run. IGF-1 was separated from its binding proteins in serum prior to measurement to obtain total IGF-1. The IGF-1: IGFBP-3 molar ratio, an indicator of bioactive IGF-1, was calculated using the following conversion factors: 1 ng/ml IGF-1 = 0.130 nm IGF-1, and 1 ng/ml IGFBP-3 = 0.036 nm IGFBP-3. Fasting insulin was measured using the DSL-10-1600 ACTIVE Insulin ELISA Kit (Diagnostic Systems Laboratories, Inc., Webster, TX). Serum was also used to study serum-stimulated growth and apoptosis of androgen dependent LNCaP cell line (27). Serum PSA was measured at Memorial Sloan-Kettering Cancer Center prospectively by a heterogeneous sandwich magnetic separation assay with the Immuno 1 System. Testosterone was measured by a competitive immunoassay with an Immulite® automated analyzer. All serum markers were measured at baseline and 1 yr.

Statistical Analysis

Independent samples *t*-tests tested for equivalency between experimental groups at baseline. Experimental group differences in changes from baseline to one year in soy isoflavone and protein intakes (total, animal, and vegetable), IGF-1, IGF binding proteins, the IGF-1: IGFBP-3 molar ratio, and fasting insulin were analyzed using analysis of variance for repeated measures, with experimental group as a between subjects factor and time as a repeated factor. Cross-sectional relationships at baseline (entire sample) between protein intake, exercise, weight, and age, to outcomes including the IGF axis (IGF-1, IGFBP-1, IGFBP-2, and IGFBP-3, and the IGF-1: IGFBP-3 molar ratio), fasting insulin, LNCaP cell growth and apoptosis, PSA and testosterone were analyzed using Pearson correlations (due to low intake at baseline, soy isoflavones were not included in the analyses). We also repeated these analyses including soy isoflavones intake on data obtained at 1 yr. Furthermore, Pearson correlations were used to evaluate the relationship between changes in these variables over 1 yr for the intervention group participants. Additionally, using a median split on protein intake, outcomes for the intervention group patients with high

Downloaded by [University of California San Francisco] at 09:41 13 October 2011

Table 1. Participant Characteristics at Baseline[1]

	Intervention ($n = 44$)	Control ($n = 49$)	P-value
Dietary Variables[2]			
Total Protein (g)	81 ± 26	79 ± 23	0.61
Animal Protein (g)	40 ± 23	40 ± 22	0.95
Vegetable Protein (g)	41 ± 18	38 ± 22	0.59
Total Isoflavones (mg)	19 ± 29	16 ± 37	0.70
Isoflavones from diet (mg)	17 ± 26	15 ± 36	0.72
Isoflavones from Suppl. (mg)	1 ± 5	1 ± 4	0.71
IGF axis[3]			
IGF-1 (ng/ml)	168 ± 64	153 ± 62	0.29
IGFBP-1 (ng/ml)	30 ± 22	26 ± 18	0.35
IGFBP-2 (ng/ml)	459 ± 246	498 ± 215	0.45
IGFBP-3 (ng/ml)	1792 ± 365	1699 ± 386	0.27
IGF-1 : IGFBP-3 (molar ratio)	0.35 ± 0.17	0.32 ± 0.12	0.39
Insulin Levels[4]			
Fasting Insulin (μIU/ml)	6.8 ± 5	7.6 ± 7	0.50

1: Abbreviations are as follows: IGF-1, insulin-like growth factor 1; IGFBP, insulin-like growth factor binding protein; μIU, micro-International units; FBS, fetal bovine serum; PSA, prostate specific antigen. 2: $n = 42$ for intervention group and $N = 43$ for control group. 3: $n = 40$ for intervention group and $N = 40$ for control group. 4: $n = 39$ for intervention group and $N = 40$ for control group.

protein intake at 1 yr were compared to outcomes of those with low protein intake using independent samples t-tests.

Results

Baseline characteristics of experimental and control group have been described in detail previously (27,29); baseline 3-day diet diary, IGF axis, and fasting insulin data are presented in Table 1. No significant differences were observed between the two groups at baseline in diet, IGF axis or fasting insulin variables (one patient's fasting insulin level of 221 μIU/ml, far greater than 3 standard deviations from mean, was not included).

Baseline correlations of protein intake, exercise, weight and age, to prostate cancer markers in the entire sample are seen in Table 2. Higher age was associated with decreased IGF-1, IGF-1: IGFBP-3 molar ratio, and lower LNCaP cell growth. Higher levels of exercise were associated with lower IGF-I and IGF-1: IGFBP-3 molar ratio. Higher weight was associated with lower levels of IGFBP-1 and IGFBP-2 and with higher levels of fasting insulin. Higher levels of dietary protein from both animal and vegetable sources were associated with higher IGF-1: IGFBP-3 molar ratios. In addition, higher consumption of total protein was associated with lower testosterone levels, and higher consumption of animal protein was associated with higher fasting insulin and lower IGFBP-1. Higher levels of vegetable protein were also associated with increased LNCaP cell growth. In addition, at baseline, fasting insulin was negatively correlated to IGFBP-1 ($r = -.418$, $P < 0.001$) and IGFBP-2 ($r = -.332$, $P < 0.001$), but showed no relationship to IGFBP-3.

Experimental group differences in changes from baseline to one year are shown in Table 3 and Table 4. Follow-up analyses involving dietary variables are based on 74 participants (37 in each group) who had complete data at both time points (Table 3). Follow-up analyses involving IGF axis and insulin data are based on 80 participants (40 in each group) (Table 4). At 1 yr, intervention patients reported higher intakes of total and vegetable protein, lower intakes of animal protein and higher intakes of soy isoflavones, both from foods and the soy protein supplement. Patients in the control group showed no changes in these variables at 1 yr.

Significant increases in IGF-1 and IGFBP-3 from baseline to 1 yr were observed regardless of experimental grouping. Intervention patients, but not control patients, demonstrated increases in IGFBP-1 at 1 yr. There were no statistically

Table 2. Correlations of Age, Weight, Exercise, and Protein Intake with IGF Axis, Insulin Levels, and Prostate Cancer Markers at Baseline[1,2]

Variable	Age (y)	Weight (kg)	Exercise (hrs)	Total Protein (g)	Animal Protein (g)	Vegetable Protein (g)
IGF axis						
IGF-1 (ng/ml)	−0.38***	0.136	−0.22*	0.20	0.14	0.08
IGFBP-1 (ng/ml)	0.01	−0.453***	0.15	−0.16	−0.23*	0.06
IGFBP-2 (ng/ml)	0.18	−0.357**	−0.03	−0.18	−0.10	−0.11
IGFBP-3 (ng/ml)	−0.21	0.032	−0.02	−0.20	−0.09	−0.15
IGF-1:IGFBP-3 (molar ratio)	−0.27*	0.135	−0.23*	0.44***	0.27*	0.24*
Insulin Levels						
Fasting Insulin (μIU/ml)	−0.03	0.469***	0.10	0.14	0.29*	−0.16
Prostate Cancer Markers						
LNCaP apoptosis (% FBS)	−0.04	0.020	0.20	0.09	0.20	−0.13
LNCaP growth (% FBS)	−0.25*	0.205	0.02	0.12	−0.07	0.23*
PSA (ng/ml)	0.04	0.029	−0.16	0.09	0.07	0.02
Testosterone (ng/dl)	−0.02	−0.372	0.06	−0.22*	−0.21	−0.03

1: Abbreviations are as follows: IGF-1, insulin-like growth factor 1; IGFBP, insulin-like growth factor binding protein; μIU, micro-International units; FBS, fetal bovine serum; PSA, prostate specific antigen. 2: For protein data, $n = 77$ for correlations with IGF axis, LNCaP apoptosis and growth, $n = 76$ with insulin, $n = 85$ with PSA, $n = 78$ with testosterone. For age, weight, and exercise data, $n = 80$ for correlations with IGF axis, LNCaP apoptosis and growth, $n = 79$ with insulin, $n = 93$ with PSA, $n = 81$ with testosterone. Soy isoflavone data were not included due to low variability of soy isoflavone intake at baseline. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

Downloaded by [University of California San Francisco] at 09:41 13 October 2011

Table 3. Protein and Isoflavone Intake by Experimental Group and Time Period, $N = 74$

	Baseline	1 Yr	P-value Group	P-value Time	P-value Time × Group
Total protein (g/day)					
Experimental	$80_a \pm 21$[1]	$115_b \pm 35$	0.002	0.001	0.001
Control	$79_a \pm 22$	$83_a \pm 27$			
Animal protein (g/day)					
Experimental	$39_a \pm 21$	$2_b \pm 6$	0.001	0.001	0.001
Control	$39_a \pm 22$	$39_a \pm 23$			
Vegetable protein (g/day)					
Experimental	$40_a \pm 17$	$112_b \pm 36$	0.001	0.001	0.001
Control	$40_a \pm 23$	$43_a \pm 28$			
Total isoflavones (mg/day)					
Experimental	$20_a \pm 30$	$133_b \pm 61$	0.001	0.001	0.001
Control	$18_a \pm 40$	$24_a \pm 33$			
Isoflavones from diet (mg/day)					
Experimental	$18_a \pm 28$	$76_b \pm 49$	0.001	0.001	0.001
Control	$17_a \pm 39$	$22_a \pm 29$			
Isoflavones from suppl.(mg/day)					
Experimental	$2_a \pm 6$	$57_b \pm 27$	0.001	0.001	0.001
Control	$1_a \pm 4$	$2_a \pm 6$			

1: Mean ± standard deviation.

significant differences between experimental and control patients for IGFBP-2 or the IGF-1: IGFBP-3 molar ratio. Although there was a significant group by time interaction in fasting insulin, post-hoc adjustments did not reveal any significant differences between the two groups during the 1-yr intervention.

For the intervention group, there were sufficient changes in isoflavone and protein intake from baseline to one year to examine the relationship between these changes and changes in IGF axis parameters, insulin, and prostate cancer markers as well as their interrelationship (not shown). Changes in total protein consumption were highly correlated with changes in soy isoflavones (r = .70, $P < 0.001$). Increases in vegetable protein intake were associated with increases in IGFBP-1 ($P < 0.05$). No other correlations were significant. Correlations between protein intakes and biomedical variables at one year (not shown) revealed that higher intakes of total protein were associated with higher levels of IGFBP-1 ($P < 0.01$) and with lower levels of LNCaP cell growth ($P < 0.05$). Also, higher consumption of vegetable protein, in contrast with baseline correlations, was associated with higher levels of IGFBP-1 and with lower levels of LNCaP cell growth ($P < 0.001$ for both). Conversely, higher intakes of animal protein were associated with lower levels of IGFBP-1 ($P < 0.001$), higher levels of LNCaP cell growth ($P < 0.001$), higher PSA ($P < 0.05$) and higher fasting insulin ($P < 0.01$). In

Table 4. IGF Axis and Insulin Levels by Experimental Group and Time period[1,2]

	Baseline	1 Yr	P-value Group	P-value Time	P-value Time × Group
IGF-1 (ng/ml)					
Experimental	168 ± 64[3]	199 ± 83	0.16	0.001	0.28
Control	153 ± 62	170 ± 85			
IGFBP-1 (ng/ml)					
Experimental	$30_a \pm 22$	$40_b \pm 19$	0.02	0.01	0.001
Control	$26_a \pm 18$	$25_a \pm 15$			
IGFBP-2 (ng/ml)					
Experimental	459 ± 246	501 ± 255	0.52	0.09	0.71
Control	498 ± 215	526 ± 228			
IGFBP-3 (ng/ml)					
Experimental	1792 ± 365	1894 ± 378	0.32	0.001	0.69
Control	1699 ± 386	1825 ± 422			
IGF-1: IGFBP-3 (molar ratio)					
Experimental	0.35 ± 0.17	0.38 ± 0.17	0.19	0.07	0.15
Control	0.32 ± 0.12	0.33 ± 0.13			
Fasting Insulin (μIU/ml)					
Experimental	$6.8_a \pm 5$	$5.5_a \pm 4$	0.09	0.95	0.03
Control	$7.6_a \pm 7$	$8.9_a \pm 8$			

1: Abbreviations are as follows: IGF-1, insulin-like growth factor 1; IGFBP, insulin-like growth factor binding protein; IU, international units. 2: $n = 80$ for IGF axis variables (40 in the intervention group, 40 in the control group); $n = 79$ for fasting insulin (39 in the intervention group, 40 in the control group). 3: Mean ± standard deviation.

Downloaded by [University of California San Francisco] at 09:41 13 October 2011

addition, higher consumption of vegetable protein was associated with lower levels of fasting insulin ($P < 0.01$). Correlations between soy isoflavone intakes and biomedical variables at one year (not shown) revealed that higher intakes of soy isoflavones (from both diet and supplement) were associated with higher levels of IGFBP-1 ($P < 0.01$), lower levels of LNCaP cell growth ($P < 0.05$) and lower levels of fasting insulin ($P < 0.05$).

Correlations of change in serum fasting insulin levels with changes in the IGF axis (not shown) revealed a negative relationship of insulin to IGFBP-1 ($r = -.270$, $P < 0.05$) and a positive relationship of insulin to IGFBP-3 ($r = .313$, $P < 0.01$).

Comparisons of experimental patients whose protein intake at 1 yr was above the median (117 g/day) to those below the median did not reveal significant differences on any variables.

Discussion

The results of this study indicate that high dietary intakes of protein and soy isoflavones, as a measure of soy protein intake, in the context of comprehensive lifestyle changes, do not appear to be responsible for the increase in IGF-1 observed after one year in prostate care patients in both the intervention and control groups. Furthermore, our analyses indicate that, in these patients, increased consumption of vegetable protein was associated with increased levels of IGFBP-1.

Analyses of baseline data show that this population reported a similar intake of protein (16% of energy) as typically consumed by Americans in the same age group (31). Soy isoflavone intake was minimal, as usually seen in Western diets (32). As expected, higher levels of exercise were associated with lower circulating IGF-1 (6) and lower IGF-1: IGFBP-3 molar ratio. We also found that, at baseline, dietary intake of protein from all sources was positively correlated with the IGF-1: IGFBP-3 molar ratio, in agreement with other observational studies (9,12). Furthermore, we observed higher levels of vegetable protein to be associated with increased LNCaP cell growth. This association was not evident when considering changes in these variables from baseline to 1 yr and was reversed in analyses on 1-yr data. That is, an inverse association between vegetable protein consumption and LNCaP cell growth was observed. Thus, this relationship needs to be further investigated.

As was expected with the adoption of a vegan diet supplemented with soy protein, intervention group participants reported significantly increased intakes of total and vegetable protein and decreased intake of animal protein after 1 yr. As all experimental participants were asked to add one to 3 servings of soy products and to include a 58-g serving of a soy protein supplement daily, soy isoflavone intake also significantly increased, equally from dietary sources and the soy protein supplement. In fact, the increases in protein intake from baseline to 1 yr in the experimental group were highly correlated with increases in soy isoflavones. The daily soy isoflavone levels achieved by this sample (133 mg/day) significantly exceed the average daily intake in Japanese, which has been reported to range from 30 to 50 mg/day (33).

Interventions similar to the one used in this study, including a diet providing <10% energy from fat and moderate amounts of protein (15–20% energy) together with ~60 minutes of daily aerobic exercise (the Pritikin program), have been shown to decrease IGF-1 levels in overweight men in the short-term (11 days) and long-term (14 yr) (5,6). In contrast, in the present study, IGF-1 significantly increased in both experimental and control groups, while remaining, on average, within the normal range (34). It is possible that the considerable increase in total protein (30%) in the experimental group, from an average of 80 g/day (16% of total energy) to 115 g/day, (20% of total energy), may have mitigated the potential IGF-1 lowering effect of the very-low fat diet and increased exercise. The protein level consumed by experimental participants was noticeably higher than the dietary reference intake (DRI) for adult men, which for this group would amount to 60–72 g protein/day (based on 0.8–0.96 g protein/kg body weight for omnivores-vegans) (35,36). Nonetheless, the somewhat smaller, but statistically significant, rise in IGF-1 in the control group remains unexplained. Although patients with prostate cancer show levels of IGF-1 that are approximately 8% higher than men without prostate cancer (37), we are not aware of any studies describing the progression of circulating IGF-1 over time in patients with early-stage prostate cancer. In addition, although an association between IGF-1 and risk of prostate cancer has been observed, the predictive value of plasma concentrations of IGF-1 and its binding proteins in the prognosis of prostate cancer is still being debated (4,37–40).

The level of dietary protein achieved by experimental patients at 1 yr (115 g/day) is similar to the highest quintile of protein intake in the Health Professionals Follow-Up study (107 g/day) (9). Although our dietary analysis did not allow us to distinguish soy protein from vegetable protein, it is reasonable to conclude, from the high correlation between changes in protein and soy isoflavone intake from baseline to 1 yr, that most of the protein increase that incurred in the intervention group was due to the addition of soy protein, both from soy products and from the supplement. Interestingly, Allen (12) observed that soy protein intake was positively correlated to IGF-1 in vegans, whereas plant protein from sources other than soy was inversely correlated to IGF-1, confirming previous findings that protein high in essential amino acids is an important determinant of circulating IGF-1 (15). Indeed, the food groups associated with higher levels of IGF-1 are milk, dairy products, fish, poultry, and red meat in omnivorous populations (9–11) and soymilk in vegans (12), all sources of protein high in essential amino acids. Observational studies have also indicated that intake of soy is associated with higher IGF-1 levels in men, although not in women (22), possibly because of gender differences in endogenous estrogen levels (32). It is important to note that although soy protein increases serum

Downloaded by [University of California San Francisco] at 09:41 13 October 2011

IGF-1 (23,24), randomized controlled trials using both an intact soy protein and soy protein deprived of isoflavones have demonstrated that the isoflavone content of soy is not responsible for IGF-1-raising effect of soy protein (24,25).

The finding of an increase in IGFBP-3 is in disagreement with investigations showing that low-fat diets (with or without exercise), and/or soy protein supplementation do not result in any significant short-term or long-term changes in IGFBP-3 (5,26). By contrast, Gann et al. showed a small but statistically significant reduction in IGFBP-3, and an increase in the IGF-1: IGFBP-3 molar ratio, in women following a low-fat diet (< 20% energy from fat) supplemented with 40 g of a daily soy protein supplement (25). Similarly, supplementation with 40 g of soy protein for 12 mo in a predominantly male population resulted in a significant decrease in IGFBP-3, and a significant increase in serum IGF-1 and the IGF-1: IGFBP-3 molar ratio compared to baseline (24).

Our analysis showed that IGFBP-1 significantly increased in the intervention group compared to controls over the study period. Although the change in IGFBP-1 over time was modest (33%), this finding is consistent with recent data on the effect of a very low-fat diet and exercise program in men (5,6). These investigations showed that a diet very low in fat and moderate in protein, together with daily aerobic exercise, resulted in significant increases in IGFBP-1 and significant reductions in IGF-1 compared to baseline levels (5) or to a control group (6). In addition, serum-stimulated LNCaP cell growth was reduced and apoptosis in LNCaP cells incubated with post-intervention serum was increased compared to baseline levels and to controls. Interestingly, serum IGFBP-1 levels showed an inverse relationship, whereas IGF-1 showed a positive relationship, with LNCaP cell growth (5). In the PCLT, we reported a significant decrease in serum-stimulated LNCaP cell growth and a non significant increase in LNCaP cell apoptosis in the intervention group compared to controls (27).

Unlike the Pritikin program studies (5,6), we did not find a significant reduction in fasting insulin in experimental patients over the study period, possibly due to lack of statistical power. However, there was a trend towards lower fasting insulin levels in the experimental group compared to the controls. This is encouraging, as fasting insulin has been associated with increased risk of prostate cancer (41). As expected, fasting insulin was inversely correlated to IGFBP-1 at baseline and changes in fasting insulin were negatively associated to changes in IGFBP-1 at 1 yr (42).

The examination of the relationship between changes in soy isoflavone and protein intakes from baseline to one year and changes in prostate cancer markers revealed that higher intakes of vegetable protein were associated with increases in IGFBP-1. This finding is consistent with observations that vegans have higher IGFBP-1 than meat-eaters or vegetarians (12,43). In addition, the correlations performed at one year confirmed the positive association between IGFBP-1 and vegetable protein, and also showed a positive association with soy isoflavones. Furthermore, both vegetable protein and soy isoflavones were inversely related to LNCaP cell growth, suggesting that consumption of vegetable protein and isoflavones may be beneficial against tumor growth (18–20).

Our study is limited by the post-hoc design, which did not allow us to analyze the relationship of dietary protein and isoflavones alone to the IGF axis. Due to the presence of multiple components in this intervention, we cannot rule out the influence of changes other than protein and isoflavone intake to the IGF axis. Nonetheless, the observed group differences in protein and isoflavone intakes are substantial (44% and 565% increase in protein and isoflavones, respectively, in the experimental group, compared to no change in the control group). In addition, our population represents a unique group of patients with early-stage prostate cancer who elected active surveillance, which may not allow the results of our study to be generalized to all men with prostate cancer. Another limitation is the lack of measurement of plasma isoflavones as evidence of compliance with the diet protocol, and of plasma steroid hormones other than testosterone that could have provided additional explanation for our results.

In summary, comprehensive lifestyle changes including a very low-fat vegan diet supplemented with soy protein did not result in a significantly higher rise in IGF-1 in prostate cancer patients randomized to the experimental group compared to controls. Contrary to our expectations, we did not find that either dietary protein or soy isoflavones, as an indicator of soy protein consumption, were significantly related to IGF-1. Nonetheless, given the recent findings that protein rich in essential amino acids (animal and soy protein) is associated with increases in IGF-1, it may be prudent for men with early stage prostate cancer consuming a vegan diet already adequate in dietary protein, not to exceed the protein recommendations set by the Institute of Medicine (35).

Acknowledgments and Notes

Supported by the Department of Defense Uniformed Services University Grant MDA905-99-1-0003 via the Henry M. Jackson Foundation Grant 600-06971000-236 Department of the Army (U.S. Army Medical Research Acquisition Activity W81XWH-05-1-0375-P0001), the Department of Health and Human Services (Health Resources and Services Administration #4 C76HF00803-01-01), The Prostate Cancer Foundation, National Institutes of Health 5P50CA089520-02 University of California–San Francisco Prostate Cancer Specialized Program of Research Excellence, the Safeway Foundation, and the Walton Family Foundation. Representatives Nancy Pelosi and John Murtha, and Senators Arlen Specter and Ted Stevens provided support. Insulin and IGF proteins analyses were done by Dr. Rusty Nicar at Diagnostic System Laboratory. We thank Colleen Kemp for her helpful contribution to this study, and The Solae Company, formerly DuPont Technologies, for providing the soy protein powdered beverage. Address correspondence to Gerdi Weidner, Preventive Medicine Research Institute, 900 Bridgeway, Sausalito, CA 94965. E-mail: antonella.dewell@pmri.org.

Submitted 18 May 2006; accepted in final form 9 January 2007.

References

1. Djavan B, Waldert M, Seitz C, and Marberger M: Insulin-like growth factors and prostate cancer. *World J Urol* **19,** 225–233, 2001.

Downloaded by [University of California San Francisco] at 09:41 13 October 2011

Downloaded by [University of California San Francisco] at 09:41 13 October 2011

2. Yu H and Rohan T: Role of the insulin-like growth factor family in cancer development and progression. *J Natl Cancer Inst* **92,** 1472–1489, 2000.
3. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, et al.: Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. *Lancet* **363,** 1346–1353, 2004.
4. Chan JM, Stampfer MJ, Ma J, Gann P, Gaziano JM, et al.: Insulin-like growth factor-I (IGF-I) and IGF binding protein-3 as predictors of advanced-stage prostate cancer. *J Natl Cancer Inst* **94,** 1099–1106, 2002.
5. Ngo TH, Barnard RJ, Tymchuk CN, Cohen P, and Aronson WJ: Effect of diet and exercise on serum insulin, IGF-I, and IGFBP-1 levels and growth of LNCaP cells in vitro (United States). *Cancer Causes Control* **13,** 929–935, 2002.
6. Barnard RJ, Ngo TH, Leung PS, Aronson WJ, and Golding LA: A low-fat diet and/or strenuous exercise alters the IGF axis in vivo and reduces prostate tumor cell growth in vitro. *Prostate* **56,** 201–206, 2003.
7. Chan JM, Stampfer MJ, Giovannucci E, Gann PH, Ma J, et al.: Plasma insulin-like growth factor-I and prostate cancer risk: a prospective study. *Science* **279,** 563–566, 1998.
8. Chokkalingam AP, Pollak M, Fillmore CM, Gao YT, Stanczyk FZ, et al.: Insulin-like growth factors and prostate cancer: a population-based case-control study in China. *Cancer Epidemiol Biomarkers Prev* **10,** 421–427, 2001.
9. Giovannucci E, Pollak M, Liu Y, Platz EA, Majeed N, et al.: Nutritional predictors of insulin-like growth factor I and their relationships to cancer in men. *Cancer Epidemiol Biomarkers Prev* **12,** 84–89, 2003.
10. Holmes MD, Pollak MN, Willett WC, and Hankinson SE: Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. *Cancer Epidemiol Biomarkers Prev* **11,** 852–861, 2002.
11. Larsson SC, Wolk K, Brismar K, and Wolk A: Association of diet with serum insulin-like growth factor I in middle-aged and elderly men. *Am J Clin Nutr* **81,** 1163–1167, 2005.
12. Allen NE, Appleby PN, Davey GK, Kaaks R, Rinaldi S, et al.: The associations of diet with serum insulin-like growth factor I and its main binding proteins in 292 women meat-eaters, vegetarians, and vegans. *Cancer Epidemiol Biomarkers Prev* **11,** 1441–1448, 2002.
13. Thissen JP, Ketelslegers JM, and Underwood LE: Nutritional regulation of the insulin-like growth factors. *Endocr Rev* **15,** 80–101, 1994.
14. Smith WJ, Underwood LE, and Clemmons DR: Effects of caloric or protein restriction on insulin-like growth factor-I (IGF-I) and IGF-binding proteins in children and adults. *J Clin Endocrinol Metab* **80,** 443–449, 1995.
15. Clemmons DR, Seek MM, and Underwood LE: Supplemental essential amino acids augment the somatomedin-C/insulin-like growth factor I response to refeeding after fasting. *Metabolism* **34,** 391–395, 1985.
16. Phillips LS, Harp JB, Goldstein S, Klein J, and Pao CI: Regulation and action of insulin-like growth factors at the cellular level. *Proc Nutr Soc* **49,** 451–458, 1990.
17. Sim HG and Cheng CW: Changing demography of prostate cancer in Asia. *Eur J Cancer* **41,** 834–845, 2005.
18. Zhou JR, Gugger ET, Tanaka T, Guo Y, Blackburn GL, et al.: Soybean phytochemicals inhibit the growth of transplantable human prostate carcinoma and tumor angiogenesis in mice. *J Nutr* **129,** 1628–1635, 1999.
19. Zhou JR, Yu L, Zhong Y, Nassr RL, Franke AA, et al.: Inhibition of orthotopic growth and metastasis of androgen-sensitive human prostate tumors in mice by bioactive soybean components. *Prostate* **53,** 143–153, 2002.
20. Lamartiniere CA, Cotroneo MS, Fritz WA, Wang J, Mentor-Marcel R, et al.: Genistein chemoprevention: timing and mechanisms of action in murine mammary and prostate. *J Nutr* **132,** 552S–558S, 2002.
21. Jarred RA, Keikha M, Dowling C, McPherson SJ, Clare AM, et al.: Induction of apoptosis in low to moderate-grade human prostate carcinoma by red clover-derived dietary isoflavones. *Cancer Epidemiol Biomarkers Prev* **11,** 1689–1696, 2002.
22. Probst-Hensch NM, Wang H, Goh VH, Seow A, Lee HP, et al.: Determinants of circulating insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations in a cohort of Singapore men and women. *Cancer Epidemiol Biomarkers Prev* **12,** 739–746, 2003.
23. Khalil DA, Lucas EA, Juma S, Smith BJ, Payton ME, et al.: Soy protein supplementation increases serum insulin-like growth factor-I in young and old men but does not affect markers of bone metabolism. *J Nutr* **132,** 2605–2608, 2002.
24. Adams KF, Newton KM, Chen C, Emerson SS, Potter JD, et al.: Soy isoflavones do not modulate circulating insulin-like growth factor concentrations in an older population in an intervention trial. *J Nutr* **133,** 1316–1319, 2003.
25. Gann PH, Kazer R, Chatterton R, Gapstur S, Thedford K, et al.: Sequential, randomized trial of a low-fat, high-fiber diet and soy supplementation: effects on circulating IGF-I and its binding proteins in premenopausal women. *Int J Cancer* **116,** 297–303, 2005.
26. Spentzos D, Mantzoros C, Regan MM, Morrissey ME, Duggan S, et al.: Minimal effect of a low-fat/high soy diet for asymptomatic, hormonally naive prostate cancer patients. *Clin Cancer Res* **9,** 3282–3287, 2003.
27. Ornish D, Weidner G, Fair WR, Marlin R, Pettengill EB, et al.: Intensive lifestyle changes may affect the progression of prostate cancer. *J Urol* **174,** 1065–1069; discussion 1069–1070, 2005.
28. Dunn-Emke SR, Weidner G, Pettengill EB, Marlin RO, Chi C, et al.: Nutrient adequacy of a very low-fat vegan diet. *J Am Diet Assoc* **105,** 1442–1446, 2005.
29. Daubenmier JJ, Weidner G, Marlin R, Crutchfield L, Dunn-Emke S, et al.: Lifestyle and health-related quality of life of men with prostate cancer managed with active surveillance. *Urology* **67,** 125–130, 2006.
30. Ornish DM, Lee KL, Fair WR, Pettengill EB, and Carroll PR: Dietary trial in prostate cancer: Early experience and implications for clinical trial design. *Urology* **57,** 200–201, 2001.
31. Wright JD, Wang CY, Kennedy-Stephenson J, and Ervin RB: Dietary intake of ten key nutrients for public health, United States: 1999–2000. *Adv Data* 1–4, 2003.
32. Vrieling A, Voskuil DW, Bueno de Mesquita HB, Kaaks R, van Noord PA, et al.: Dietary determinants of circulating insulin-like growth factor (IGF)-I and IGF binding proteins 1, -2, and -3 in women in the Netherlands. *Cancer Causes Control* **15,** 787–796, 2004.
33. Wakai K, Egami I, Kato K, Kawamura T, Tamakoshi A, et al.: Dietary intake and sources of isoflavones among Japanese. *Nutr Cancer* **33,** 139–145, 1999.
34. Yu H, Mistry J, Nicar MJ, Khosravi MJ, Diamandis A, et al.: Insulin-like growth factors (IGF-I, free IGF-I and IGF-II) and insulin-like growth factor binding proteins (IGFBP-2, IGFBP-3, IGFBP-6, and ALS) in blood circulation. *J Clin Lab Anal* **13,** 166–172, 1999.
35. Food and Nutrition Board Institute of Medicine: Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients). Washington, DC: The National Academy Press, 2002.
36. Position of the American Dietetic Association and Dietitians of Canada: Vegetarian diets. *J Am Diet Assoc* **103,** 748–765, 2003.
37. Cohen P: Serum insulin-like growth factor-I levels and prostate cancer risk–interpreting the evidence. *J Natl Cancer Inst* **90,** 876–879, 1998.
38. Harman SM, Metter EJ, Blackman MR, Landis PK, and Carter HB: Serum levels of insulin-like growth factor I (IGF-I), IGF-II, IGF-binding protein-3, and prostate-specific antigen as predictors of clinical prostate cancer. *J Clin Endocrinol Metab* **85,** 4258–4265, 2000.
39. Yu H, Nicar MR, Shi R, Berkel HJ, Nam R, et al.: Levels of insulin-like growth factor I (IGF-I) and IGF binding proteins 2 and 3 in serial postoperative serum samples and risk of prostate cancer recurrence. *Urology* **57,** 471–475, 2001.
40. Janssen JA, Wildhagen MF, Ito K, Blijenberg BG, Van Schaik RH, et al.: Circulating free insulin-like growth factor (IGF)-I, total IGF-I, and IGF binding protein-3 levels do not predict the future risk to develop prostate cancer: results of a case-control study involving 201 patients within a population-based screening with a 4-year interval. *J Clin Endocrinol Metab* **89,** 4391–4396, 2004.

41. Hsing AW, Chua S, Jr., Gao YT, Gentzschein E, Chang L, et al.: Prostate cancer risk and serum levels of insulin and leptin: a population-based study. *J Natl Cancer Inst* **93,** 783–789, 2001.
42. Suikkari AM, Koivisto VA, Rutanen EM, Yki-Jarvinen H, Karonen SL, et al.: Insulin regulatesthe serum levels of low molecular weight insulin-like growth factor-binding protein. *J Clin Endocrinol Metab* **66,** 266–272, 1988.
43. Allen NE, Appleby PN, Davey GK, and Key TJ: Hormones and diet: low insulin-like growth factor-I but normal bioavailable androgens in vegan men. *Br J Cancer* **83,** 95–97, 2000.

Downloaded by [University of California San Francisco] at 09:41 13 October 2011