Changes in prostate gene expression in men undergoing an intensive nutrition and lifestyle intervention

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Epidemiological and prospective studies indicate that comprehensive lifestyle changes may modify the progression of prostate cancer. However, the molecular mechanisms by which improvements in diet and lifestyle might affect the prostate microenvironment are poorly understood. We conducted a pilot study to examine changes in prostate gene expression in a unique population of men with low-risk prostate cancer who declined immediate surgery, hormonal therapy, or radiation and participated in an intensive nutrition and lifestyle intervention while undergoing careful surveillance for tumor progression. Consistent with previous studies, significant improvements in weight, abdominal obesity, blood pressure, and lipid profile were observed (all $P < 0.05$), and surveillance of low-risk patients was safe. Gene expression profiles were obtained from 30 participants, pairing RNA samples from control prostate needle biopsy taken before intervention to RNA from the same patient's 3-month postintervention biopsy. Quantitative real-time PCR was used to validate array observations for selected transcripts. Two-class paired analysis of global gene expression using significance analysis of microarrays detected 48 up-regulated and 453 down-regulated transcripts after the intervention. Pathway analysis identified significant modulation of biological processes that have critical roles in tumorigenesis, including protein metabolism and modification, intracellular protein traffic, and protein phosphorylation (all $P < 0.05$). Intensive nutrition and lifestyle changes may modulate gene expression in the prostate. Understanding the prostate molecular response to comprehensive lifestyle changes may strengthen efforts to develop effective prevention and treatment. Larger clinical trials are warranted to confirm the results of this pilot study.

exercise | lifestyle changes | prostate cancer | SHOC2 | stress management

Epidemiological evidence (1, 2) and migrant studies (3) indicate that the incidence of clinically significant prostate cancer is much lower in parts of the world where people eat a predominantly low-fat, plant-based diet. We (4, 5) and others (6) have shown previously that diet and lifestyle interventions in men with early-stage prostate cancer decrease prostate-specific antigen (PSA) and decrease the rate of PSA increase. These studies provided some evidence that comprehensive lifestyle changes may have therapeutic potential in early prostate cancers. However, although these interventions are associated with decreased circulating insulin-like growth factor 1 (IGF1) (7), and although serum from men after intervention has reduced the ability to stimulate prostate cell-line growth *in vitro* (4), the actual molecular effects of these interventions in prostate tissue have not been previously examined.

Many men with indolent prostate cancers detected by PSA screening will not exhibit disease progression during their lifetime; their treatment and associated side effects are unnecessary (8). We report here the results of the Gene Expression Modulation by Intervention with Nutrition and Lifestyle (GEMINAL) study, a prospective single-arm pilot clinical intervention study in men with indolent low-risk prostate cancers, defined by strict clinical and pathologic criteria designed to minimize the risk for metastatic disease as a result of study participation (9). The 30 men who enrolled did not undergo surgery or radiation therapy to treat their low-risk tumors; rather, they underwent comprehensive lifestyle changes (low-fat, whole-foods, plant-based nutrition; stress management techniques; moderate exercise; and participation in a psychosocial group support). Participants donated serial prostate needle biopsies at baseline and after 3 months of the lifestyle intervention, from which nanogram quantities of mRNA were purified. At the time this clinical trial began, commercial expression array platforms were not sensitive to nanogram RNA quantities. Therefore, a reproducible linear RNA amplification and printed cDNA array platform was used, as in our previous studies of melanoma (10), where subsequent studies have confirmed the validity of the gene expression findings (11, 12). Furthermore, quantitative real-time PCR (QRT-PCR) was used to provide initial confirmation of the study results, comparing in pairwise fashion each man's postintervention to his own preintervention sample. This article examines the relationship of comprehensive diet and lifestyle changes to gene expression in the prostate.

Results

In the GEMINAL study, 273 men were screened, 96 declined to participate, 146 did not meet inclusion criteria, and 31 were enrolled. The participants' demographics included a mean age of 62.3 years (range 49–80) and a mean PSA level of 4.8 ng/ml (range 0.5–21.4) on the day of the initial biopsy. As expected from the trial eligibility criteria, all patients had a Gleason score of 6. Eighty-four percent of the men identified their ethnicity as Caucasian, 9% Hispanic, 3% Asian, and 4% African-American. Two-thirds of the men were married, and 72% were currently employed.

Trial eligibility required PSA $\leq$10 [or PSA $\leq$15 if the patient had a history of benign prostatic hyperplasia (BPH)] at the time of screening. One outlier patient with a history of BPH and a prostate volume of 90 cc, as assessed by transrectal ultrasound, had screening

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Conflict of interest statement: Although none of the following is a true conflict of interest, in the spirit of full disclosure, D.O. writes general-interest books on preventive medicine, receives lecture honoraria, and consults with food companies to make more healthful foods.

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Data deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE10306).

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Table 1. Cardiovascular risk factors and psychological functioning at baseline and 3 months

Variable	Baseline, Mean ± SD	3 months, Mean ± SD	Mean change, 3-month baseline	95% CI of change		*P**
				Lower	Upper	
Cardiovascular risk factors						
BMI	26.5 ± 3.6	23.9 ± 3.0	−2.6	−3.0	−2.2	<0.001
Systolic blood pressure, mmHg	129.5 ± 15.1	120.3 ± 12.6	−9.2	−13.8	−4.6	<0.001
Diastolic blood pressure, mmHg	68.2 ± 10.7	62.8 ± 10.3	−5.4	−8.4	−2.3	<0.002
LDL, mg/dl	121.7 ± 28.2	87.6 ± 25.1	−34.2	−41.6	−26.8	<0.001
HDL, mg/dl	48.5 ± 12.7	40.2 ± 12.1	−8.3	−11.5	−5.2	<0.001
Total cholesterol, mg/dl	191.7 ± 33.1	146.5 ± 31.6	−45.2	−54.4	−36.0	<0.001
LDL/HDL ratio	2.7 ± 1.0	2.3 ± 0.8	−0.4	−0.6	−0.1	<0.002
Triglycerides, mg/dl	107.0 ± 72.1	93.6 ± 46.3	−13.4	−32.3	5.5	0.158
C-reactive protein (ln)	0.12 ± 1.1	−0.14 ± 1.3	−0.26	−0.64	0.12	0.168
Psychological functioning						
SF-36						
Mental component summary	49.7 ± 11.8	56.2 ± 5.5	6.5	2.6	10.3	<0.003
Physical component summary	54.7 ± 4.9	55.4 ± 5.5	0.8	−1.5	3.0	0.485
Impact of Event Scale						
Intrusive thoughts	6.2 ± 5.6	3.7 ± 4.8	−2.5	−4.2	−0.8	<0.01
Avoidance	10.1 ± 6.8	7.1 ± 5.8	−3.0	−5.9	−0.1	<0.05

*Paired-samples *t* test, two-tailed.

PSAs of 11.6–12.1 ng/ml, meeting eligibility criteria, but had a PSA of 21.4 ng/ml on the day of initial biopsy. After the 3-month intervention, this patient's PSA decreased to 13.9 ng/ml, and his prostate volume decreased to 75 cc. All other participants had screening and initial biopsy-day PSA values of <10 ng/ml.

The study was safe, with no adverse events observed. The dosage of all concomitant medications remained stable through the 3-month assessment, with the exception of one participant whose dosage of a statin drug was reduced. One of 31 study participants was referred for surgery based on the result of his 3-month follow-up biopsy, which showed an increased tumor Gleason score of 3 + 4 compared with his baseline Gleason of 3 + 3.

As seen in Table 1, baseline body mass index (BMI), systolic blood pressure, and LDL cholesterol were all somewhat elevated. Quality of life scores were near the SF-36 highest-quartile cutoff score for physical health (>55.7) and near the lowest-quartile cutoff score for mental health (<49.9) (24). The psychological distress reported in this sample was comparable with the level of distress reported by other men diagnosed with prostate cancer (mean duration from diagnosis to study entry was 12.3 months) by using the Impact of Event scale (26).

Patients were able to adhere closely to the lifestyle recommendations. After 3 months, they reported consuming 11.6% (SD = 3.0) of fat calories per day, exercising >3.6 h per week (SD = 1.5), and practicing stress management 4.5 h per week (SD = 2.0). A significant improvement in cardiovascular disease risk factors was observed, including reductions in BMI, systolic and diastolic blood pressure, and lipids (Table 1). Waist circumference decreased from 97.2 ± 11.1 cm to 89.5 ± 9.2 cm ($P < 0.001$). Triglycerides and C-reactive protein decreased, although these trends did not reach

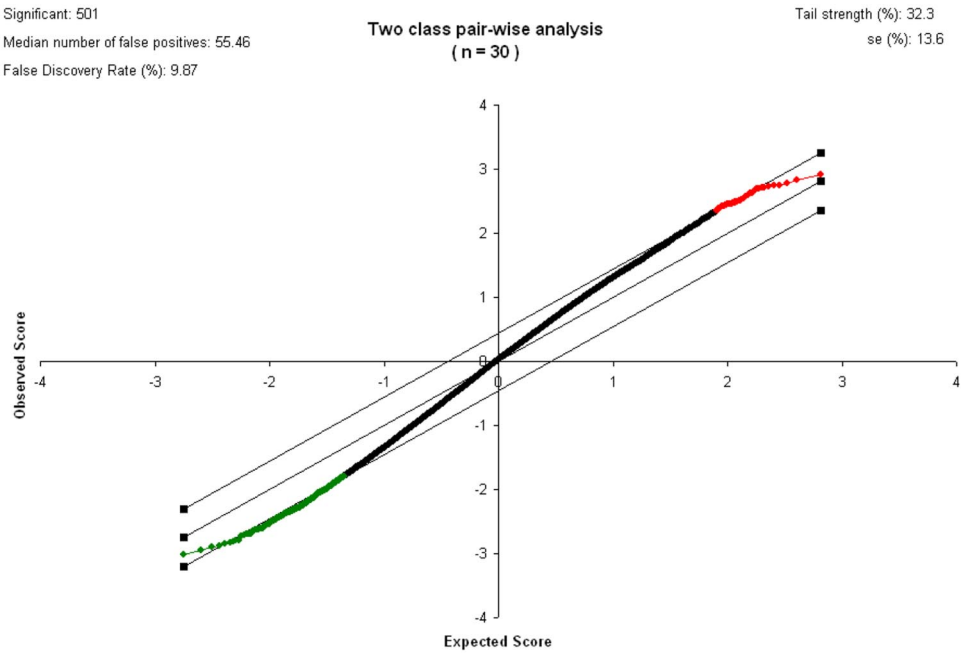

Fig. 1. Plot of two class pairwise SAM (false discovery rate of <0.10). Forty-eight transcripts were up-regulated (red) and 453 transcripts were down-regulated (green) in morphologically normal prostate after a comprehensive diet and lifestyle intervention.

Table 2. Up-regulated and down-regulated transcripts in paired pre- and post- diet/lifestyle intervention in normal prostate biopsy samples

Transcripts	SAM ranking	Unigene or genome database name
Down-regulated		
RAN	1	RAN, member RAS oncogene family
SHOC2	2	Soc-2 suppressor of clear homolog (*C. elegans*)
EST chromosome 18	3	Transcribed sequence chromosome 18
ITGA10	4	Integrin, α 10
SLC35D1	5	Solute carrier family 35 member D1
MMP9	6	Matrix metallopeptidase 9
DENND1B	7	DENN/MADD domain containing 1B
RNF150	8	Ring finger protein 150
HIPK1	9	Homeodomain interacting protein kinase 1
NUS1	12	Nuclear undecaprenyl pyrophosphate synthase 1
SBNO	13	Strawberry notch homolog 1 (*Drosophila*)
IMAGE:4610527	14	Transcribed sequence chromosome 18, 4610527
GPD1L	15	Glycerol-3-phosphate dehydrogenase 1-like
ZE03F06	16	Transcribed sequence chromosome 1, ZE03F06
KIAA0141	18	KIAA0141
ACLY	19	ATP citrate lyase
SUB1	20	SUB1 homolog (*S. cerevisiae*)
KLF6	21	Kruppel-like factor 6
CRKRS	22	Cdc2-related kinase, arginine/serine-rich
FLT1	23	Fms-related tyrosine kinase 1
Up-regulated		
NR2F1	1	Nuclear receptor subfamily 2, group F, member 1
BC029658	2	Transcribed sequence BC029658
ZNF250	3	Zinc finger protein 250
EST chromosome 8	12	Transcribed sequence chromosome 8
C21orf131	13	Chromosome 21 open reading frame 131
C11orf71	15	Chromosome 11 open reading frame 71
ZNF160	20	Zinc finger protein 160
KIAA1843	21	KIAA1843
CR627148	22	Transcribed sequence CR627148
C6orf217	29	Chromosome 6 open reading frame 217

statistical significance. Total PSA did not change significantly (from 4.8 $\pm$ 3.9 to 4.6 $\pm$ 3.4 ng/ml; $P = 0.48$), although percent free PSA was improved, from 17.5 $\pm$ 7.4 to 18.9 $\pm$ 8.3 ($P = 0.05$).

Patients reported significant reductions in psychological distress associated with prostate cancer, as indicated by lower scores on the intrusive and avoidant thoughts subscales of the Impact of Event scale. Mental health-related quality of life also improved, with increases in the Mental Component Summary score of the SF-36, although physical health-related quality of life was stable.

Each man underwent control prostate needle biopsy at baseline and an experimental biopsy after 3 months of intervention. Of the 31 patients enrolled, 30 were evaluable for gene expression, and one patient sample was excluded because the biopsy tissue did not contain sufficient prostate epithelium. The paired-specimen design, where each man provided his own control tissue, minimized the chance that interpatient differences in gene expression would obscure intervention-related differences in gene expression. In addition, each batch of RNA amplification, fluorescent cDNA labeling, and microarray hybridization contained both pre- and postintervention samples, selected randomly, to minimize the chance that these procedures would introduce artifact. Using the significance analysis of microarrays (SAM) algorithm, we detected 48 up-regulated and 453 down-regulated transcripts in normal prostate tissue after 3 months of intervention [Fig. 1, Table 2, and supporting information (SI) Dataset S1 and Dataset S2], compared with the paired normal prostate tissue samples from the same individual patients at baseline, with the false discovery rate set at 0.10. The heat map in Fig. 2 demonstrates visually the substantial modulation of gene expression seen when pre- and postintervention samples were compared.

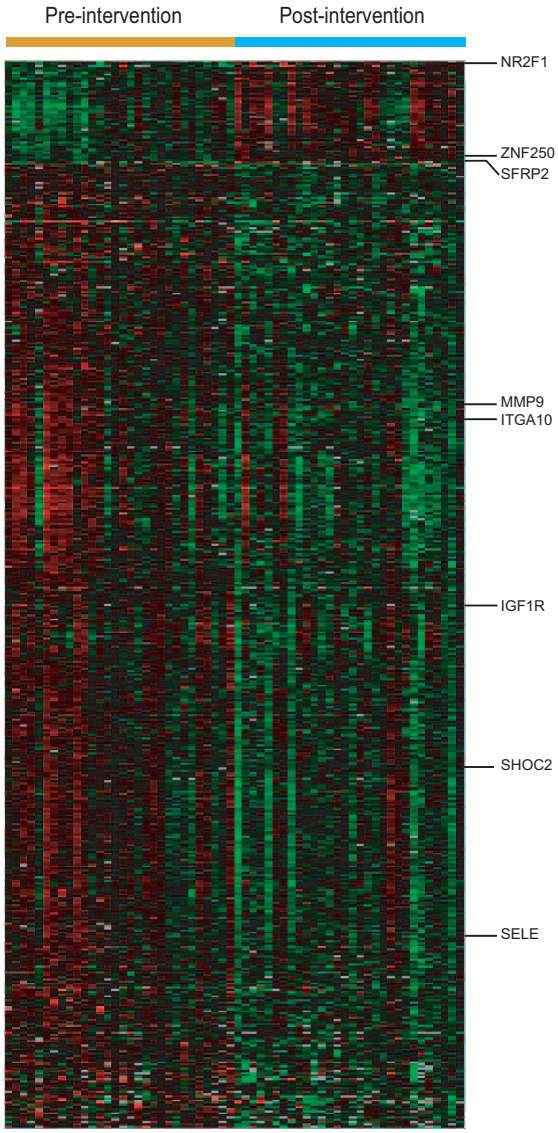

Fig. 2. Heat map of the pre- and postintervention samples demonstrating 48 up-regulated transcripts (red in the postintervention samples) and 453 down-regulated transcripts (green in the postintervention samples) in morphologically normal prostate after a comprehensive diet and lifestyle intervention.

We noted that several of the cDNA array clones in the SAM dataset remain poorly annotated in the Unigene database. Thus, we used the University of California (Santa Cruz, CA) (UCSC) genome database and software tools (23) to investigate whether these clones might represent recently recognized genome elements such as microRNAs, snoRNAs, or scaRNAs to confirm evidence that clones represent transcribed loci and to exclude clones whose sequence was composed of repetitive DNA elements. Eighteen up-regulated transcripts and 388 down-regulated transcripts met these strict criteria and are presented in Dataset S1 and Dataset S2. No matches to small RNAs in snoRNAbase (27) and miRBase (28) were found. The 10 highest ranked up-regulated and 20 highest ranked down-regulated transcripts are listed in Table 2.

For the subset of samples with sufficient total RNA remaining after array analysis, validation QRT-PCR experiments were performed to confirm the accuracy of array gene expression measurements. Results across the 30 study patients are shown in Fig. 3 comparing *SHOC2* array and QRT-PCR measurements. Both methods demonstrated transcript down-regulation after the life-

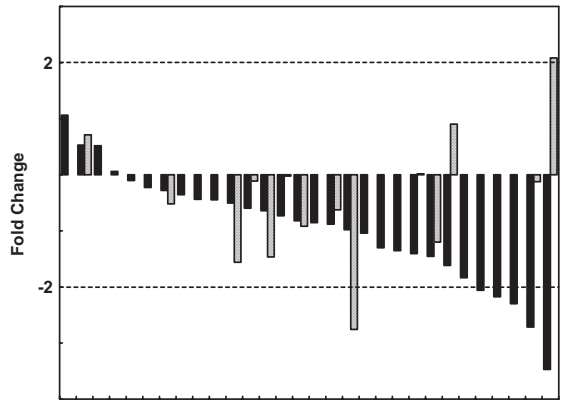

Fig. 3. Waterfall plot of ratio of log (base 2) post- versus preintervention of *SHOC2* gene expression comparing microarray (filled bars) to the mean of three independent replicate QRT-PCR (cross-hatched bars) measurements. No QRT-PCR data are shown for 14 of the 30 patients for whom insufficient needle biopsy RNA was available, precluding measurement.

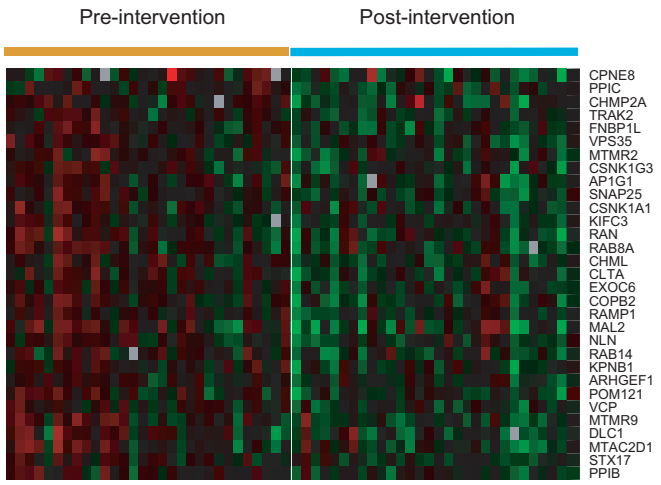

Fig. 4. Heat map of the gene ontology group ''Intracellular Protein Traffic'' illustrating the down-regulation of these 31 transcripts. Pre- and postcomprehensive diet and lifestyle intervention samples are indicated.

style intervention, and there was no statistical difference between the array and QRT-PCR measurements using the Wilcoxon matched-pairs test.

To gain further insight into the molecular changes occurring in the prostate tissue, we conducted pathway analysis using the PANTHER algorithm (21), which identified significant modulation of biological processes with critical roles in tumorigenesis among the genes down-regulated after intervention (Figs. 3 and 4, Table 3, and Dataset S3). Pathways involved in protein metabolism and modification, intracellular protein traffic, and protein phosphorylation were significantly down-regulated (all $P < 0.05$) (see Fig. 4 and Dataset S4).

Discussion

The GEMINAL study was a prospective pilot trial of diet and lifestyle intervention in men with low-risk prostate cancer. Unlike patients with other tumor types, who must undergo immediate resection, radiation therapy, or, rarely, chemotherapy, low-risk prostate cancers may be observed, providing a unique opportunity for molecular study when men undergo serial biopsy during active surveillance. Similar to previous active surveillance clinical studies (29, 30), our patients reported no adverse events. Referral for definitive surgery or radiation therapy occurred for only one patient, whose follow-up biopsy showed an increased Gleason score of prostate cancer compared with baseline (which was likely due to sampling variability in only 3 months).

Our observations provide molecular hypotheses that may help explain some of the effects of comprehensive lifestyle changes. The imperative to understand these effects has been spurred by the observation in prospective trials that healthy diet and lifestyle practices may improve recurrence-free and overall survival in colon cancer (31) and breast cancer (32).

We found a set of *RAS* family oncogenes (*RAN*, *RAB14*, and *RAB8A*) to be down-regulated. In the prostate, RAN (ras-related nuclear protein) may function as an androgen receptor coactivator, and its expression is increased in tumor tissues (33). However, despite the down-regulation of this AR coactivator, androgen-regulated PSA in our study was stable comparing baseline with 3-month measurements, both at the RNA level and in serum measurement of total PSA.

The percentage of free PSA was significantly improved when the entire group of 30 participants was analyzed. However, the predictive value of the percentage of free PSA has been validated only in men with a PSA of >4 ng/ml (34–37). As expected, because of the small sample size, our results were not statistically significant when analysis was limited to the 15 of 30 men whose PSA on biopsy day 1 was >4 ng/ml. Therefore, our percentage of free PSA results should be considered exploratory.

Future studies with a control group and/or with longer than 3-month intervention will be needed to see whether the stabilization in PSA or the percentage of PSA modulation we observed can be attributed to an effect of diet and lifestyle on androgen receptor signaling. In future diet and lifestyle intervention trials, it also would be interesting to directly examine the modulation of the activity of the androgen receptor at its target DNA response elements. For example, a trial could be designed to examine whether AR occupancy at response elements within the prostate-specific antigen promoter is modulated by diet and lifestyle intervention. RAN also is known to have effects on the control of DNA

Table 3. Overrepresented ontology categories in molecular functions and biological processes ($P < 0.05$) among genes down-regulated after a diet/lifestyle intervention

	NCBI: *Homo sapiens* genes, number of genes	GEMINAL down-regulated genes, number of genes	Expected	*P*
Molecular function				
Ligase	468	19	5.72	0.007
Ubiquitin-protein ligase	523	12	3.09	0.014
Membrane traffic protein	359	13	4.39	0.017
Select regulatory molecule	1,190	27	14.55	0.049
Biological process				
Protein metabolism and modification	3,040	69	37.8	<0.001
Intracellular protein traffic	1,008	31	12.33	<0.001
Protein modification	1,157	32	14.15	0.003
Protein phosphorylation	660	20	8.07	0.044

synthesis and cell division (38), which suggests that its down-regulation could have antitumor effects independent of its activity in modulating hormone signaling.

We validated the down-regulation of *SHOC2* by QRT-PCR. This gene encodes a protein that is essential in MAPK activation by growth factors (39), but expression levels in human tumors have not been reported previously. SHOC2 is proposed to be an attractive new target of therapeutic intervention in the treatment of the many human malignancies with up-regulated MAPK activity (39).

Several recent studies have examined how nutrition affects gene expression in the context of obesity and metabolic syndrome (40, 41). These investigations (the FUNGENUT study) profiled gene expression in subcutaneous adipose tissue and found down-regulation of IGF pathway genes and genes related to fat metabolism. Our results in prostate tissue were very similar; we also found down-regulation of IGF pathway genes [*IGF1 receptor (IGF1R), phosphoinositide-3-kinase, class 2, α-polypeptide (PIK3C2A),* and *forkhead box A2 (FOXA2)*] and down-regulation of fat metabolism genes [*acyl-CoA dehydrogenase, long chain (ACADL),* and *phytanoylCoA 2-hydroxylase (PHYH)*]. In addition, prostate tissue exhibited down-regulation of carbohydrate metabolism genes [*6-phosphofructo-2-kinase (PFKFB1), glycerol-3-phosphate dehydrogenase 1-like (GPD1L),* and *ATP citrate lyase (ACLY)*].

Recent studies also indicate that exercise may influence gene expression (42–44). These studies reported changes in gene expression in leukocytes after moderate or exhaustive exercise, with modulation of genes related to oxidative stress and inflammation. However, GEMINAL results did not include changes related to oxidative stress or inflammation. Differences in GEMINAL versus these exercise study observations may relate to lower intensities of exercise prescribed for our elderly population and tissue-specific differences in the response to exercise.

Two important challenges and opportunities in conducting research in patients with clinically localized prostate tumors were evident in our study. First, many men with low-risk tumors are reluctant to forego definitive treatment, as illustrated by our need to screen 271 men to enroll 31. Second, our analysis was limited to normal prostate tissue because tumor tissue was present on the biopsy specimens of only a minority of patients. Thus, the implications of this study are not limited to men with prostate cancer. Because of the microfocal nature of low-risk prostate tumors and the limitations of ultrasound biopsy guidance, we were unable to precisely match pre- and postintervention tumor samples for individuals in our cohort.

It is important to recognize the limitations of this study. Because of the expense of clinical investigation, it was not feasible to conduct a randomized trial in the absence of data first demonstrating that diet and lifestyle intervention may modulate gene expression. GEMINAL now provides preliminary evidence that prostate gene expression may be modulated by diet and lifestyle and provides the rationale needed to support new randomized controlled trials. Because only one-third of patient biopsies in our study included tumor tissue, we were limited to examining the response of the normal prostate tissue (stroma and epithelium) to the intervention. It will be very important for future work to examine tissue molecular responses to determine whether the normal stroma, tumor stroma, normal epithelium, tumor epithelium, or a combination of these tissues respond to diet and lifestyle changes.

Although we observed a decrease in potential oncogenes such as RAN and SHOC2, suggesting that the effects of the intervention were beneficial, confirmatory studies are needed. One level of confirmation would determine whether the RNA changes observed here are accompanied by parallel changes in protein levels. In addition, our work predicts that functional studies that lower the levels of GEMINAL down-regulated genes should reduce tumor incidence or progression in appropriate animal models.

Future investigations should enroll larger numbers of patients and should include a control group to rule out the theoretical possibility (although never documented) that repeat biopsy could introduce gene expression changes independent of an intervention. Ongoing, randomized trials of diet and lifestyle interventions in men with low-risk prostate cancer, including the Molecular Effects of Nutrition Supplements (MENS) and the Mens Eating and Living Study (MEAL) studies (45), feature control groups. These studies are expected to provide important confirmatory data when they report in future years. With improvements in commercial RNA amplification and oligonucleotide microarray platforms, future studies may adopt these standards to facilitate cross-study comparisons and to comprehensively examine the human transcriptome. The intervention used in our study was complex, and future studies may address which components or combinations of components of the intervention are needed to produce particular molecular effects. In addition, nutrients may produce different phenotypes in patients with different genotypes. For example, one P450 cytochrome allele may metabolize a dietary substrate to a bioactive form, in contrast to another allele that produces an inactive metabolite (46). As more examples of diet–gene interactions are discovered, increased power and sophistication of clinical trials will become possible.

In conclusion, the GEMINAL study suggests that intensive nutrition and lifestyle changes may modulate gene expression in the prostate. Understanding the mechanisms of how comprehensive lifestyle changes affect transcriptional regulation may strengthen efforts to develop effective prevention and treatment strategies for prostate cancer. Larger randomized controlled clinical trials are now warranted to confirm and extend the hypotheses generated by the results of this pilot study and to better understand the relative contribution of each component of the intervention.

Materials and Methods

Study Subjects and Clinical Assays. Men with low-risk prostate cancer willing to make comprehensive lifestyle changes gave informed consent under a protocol approved by the University of California San Francisco Institutional Review Board. These patients chose active surveillance rather than conventional treatments for prostate cancer for reasons unrelated to this study. Eligibility criteria included: pathology-confirmed prostate cancer, PSA $\leq$10 (or $<$15 if there was documented benign prostatic hyperplasia or prostatitis) at the time of screening, Gleason sum $\leq$6 with no pattern 4 or 5, stage T1 or T2a, $\leq$33% of biopsy cores positive, and $\leq$50% of the length of a tumor core positive. Standard clinical assays were used for waist circumference, weight, height, blood pressure, serum lipids, C-reactive protein, and PSA.

Lifestyle Intervention. A 3-month comprehensive lifestyle modification was prescribed (13, 14), comprising a 3-day intensive residential retreat, followed by an outpatient phase where participants were in weekly telephone contact with a study nurse. Lifestyle modifications included a low-fat (10% of calories from fat), whole-foods, plant-based diet, stress management 60 min per day (gentle yoga-based stretching, breathing, meditation, imagery, and progressive relaxation), moderate aerobic exercise (walking 30 min per day for 6 days per week), and a 1-h group support session per week. The diet was supplemented with soy (1 daily serving of tofu plus 58 g of a fortified soy protein powdered beverage), fish oil (3 g daily), vitamin E (100 units daily), selenium (200 mg daily), and vitamin C (2 g daily). Participants were provided with all of their food during the intervention period. A registered dietitian, exercise physiologist, clinical psychologist, nurse, and stress management instructor were available for education and counseling.

Biopsy Processing. The 18-gauge core needle biopsies were collected, under ultrasound guidance, from each patient before and after the intervention and frozen in OCT medium over dry ice. Then 14-μm cryostat sections were processed for H&E staining and cytokeratin immunohistochemistry (cat. no. M0630; DAKO; loss of cytokeratin identifies prostate tumor tissue) (15), and 17 sections were used for RNA preparation on RNEasy columns (Qiagen) guided by the study pathologist. Only areas of normal prostate peripheral-zone tissue containing both stroma and epithelial cells were dissected and processed for RNA.

RNA Amplification, Labeling, and Arrays. Briefly, 25–100 ng of total RNA, side-by-side with an equal quantity of universal human reference RNA (cat. no. 740000; Stratagene), was linearly amplified through two rounds of modified *in vitro* transcription (16). Randomized amplified RNAs were converted to aminoallyl-modified cDNA, coupled to *N*-hydroxysuccinimidyl esters of Cy3 or Cy5 (Amersham Biosciences) (17), and hybridized to arrays compriing 20,862 cDNAs (Research Genetics) at 63°C for 12–16 h (18). Slides were then washed and immediately scanned and analyzed with

Axon Imager 4000b (Axon Instruments) using GENEPIXPRO3.0. Strategies to minimize the risk of artifact included randomizing samples from pre- and postintervention to linear amplification batches and randomizing hybridizations among the two array batches used in these experiments.

Microarray Data Analysis and Bioinformatics. Gene expression was analyzed with CLUSTER (19) by using the average linkage metric. Log-transformed (base 2) median of ratio values were subjected to LOESS normalization by using MARRAY in the R environment (20) and filtered for genes where data were present in 80% of experiments. The resulting data were then analyzed by using the SAM package. A two-class paired analysis was used to compare pre- and postintervention gene expression data. Genes were considered significant if the q value was <0.10. (A q value estimates the probability of a true change at follow-up when the false discovery rate is $<10\%$.) The heat map in Fig. 1*B* was generated by using SAMSTER (http://falkow.stanford.edu/whatwedo/software/programs/samster.pdf).

Identification of ontology groups (biological processes/molecular functions) and pathways were conducted by using PANTHER Version 6.0 (www.pantherdb.org) (21). The *Homo sapiens* gene list of the National Center for Biotechnology Information was chosen as the reference list for comparison using the χ^2 test for a single proportion (binomial outcome) with a Bonferroni correction for multiple testing ($P < 0.05$ was considered significant).

Clone annotation was updated by using Unigene Build no. 208 of the human genome accessed through the SOURCE database (22) and UCSC genome database and software tools (23) for clones without Unigene annotation. Clones with a DNA sequence corresponding to repetitive elements or that represented nontranscribed DNA were removed from the final dataset.

QRT-PCR Analysis. First, 100 ng of total RNA was reverse-transcribed by using the iScript kit (Bio-Rad). Then, 5 ng of cDNA was subjected to QRT-PCR using Applied Biosystems (ABI) TaqMan assays (validated for each gene) on the ABI 7900HT instrument. Data were analyzed by the $\Delta\Delta$Ct method (ABI), which provides the target gene expression value as unitless fold changes in the unknown sample compared with a calibrator sample; both unknown and calibrator sample target gene expression data were normalized by the relative expression of a housekeeping gene, GUSB, β-glucuronidase. GUSB was chosen because it displayed the lowest variability among the three genes (GUSB, cyclophilin A, and β-actin) tested in sets of normal prostate cDNA. Fold change was used to compare the two methods, array and QRT-PCR, evaluating change in gene expression from pre- to postintervention.

Quality of Life and Psychological Distress. The SF-36 (QualityMetric), a survey instrument well validated in the prostate cancer population (24), was administered to men at baseline and after the 3-month intervention. Psychological distress was assessed by using the Impact of Event scale (25), a well validated measure of distress associated with a traumatic event, such as a cancer diagnosis.

Statistics. By using the paired data, the mean fold change between QRT-PCR and array results was analyzed with paired t tests, and the Wilcoxon matched-pairs test was conducted. Descriptive statistics were provided for clinical variables age, ethnicity/race, PSA, serum lipid values, and quality of life.

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