

Short Communication

Low-Fat, Low-Glycemic Load Diet and Gene Expression in Human Prostate Epithelium: A Feasibility Study of Using cDNA Microarrays to Assess the Response to Dietary Intervention in Target Tissues

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Abstract

Purpose: We examined the feasibility of using gene expression changes in human prostate epithelium as a measure of response to a dietary intervention.

Materials and Methods: Eight men with newly diagnosed prostate cancer were randomized to a low-fat/low-glycemic load intervention arm (<20% energy from fat and total daily glycemic load <100) or a "standard American" control arm (\approx 35% energy from fat and total daily glycemic load >200). Prostate tissue was collected before randomization and \sim 6 weeks later, at the time of radical prostatectomy. Epithelium was acquired by laser capture microdissection, and transcript abundance levels were measured by cDNA microarray hybridization and confirmed by quantitative reverse transcription-PCR.

Results: Men in the intervention arm consumed 39% less total energy ($P = 0.004$) and the difference in

weight change between intervention and control arms was -6.1 kg ($P = 0.02$). In the intervention arm, 23 (0.46%) of 5,711 cDNAs with measurable expression were significantly altered ($P < 0.05$; false discovery rate, $\leq 10\%$). In the control arm, there were no significant changes in transcript expression, even when using a false discovery rate as high as 50%.

Conclusions: A 6-week, low-fat/low-glycemic load diet was associated with significant gene expression changes in human prostate epithelium. These results show the feasibility of using prostate tissues collected at diagnosis and at surgery to study the effects of dietary manipulation on prostate tissue, which may give insight into the molecular mechanisms underlying the associations of diet and obesity with the development or progression of prostate cancer. (Cancer Epidemiol Biomarkers Prev 2007;16(10):2150-4)

Introduction

There is good evidence that obesity and perhaps, a high fat intake, due to its strong correlation with obesity, increase the risk of high-grade or aggressive prostate cancer, prostate cancer mortality, and the risk of poor outcome following diagnosis and treatment (1-3). However, the molecular mechanisms underlying these associations remains largely unknown. Experimental animal and *in vitro* studies have shown that dietary patterns and

food constituents can affect gene expression, protein synthesis, cell signaling, and other important events related to carcinogenesis (4-6). In humans, however, it is difficult to test hypotheses related to the mechanisms and pathways that underlie diet and cancer associations because appropriate tissues are rarely available. Human studies are generally limited to measuring circulating biomarkers, such as serum steroid hormones, which are influenced by diet and are associated with carcinogenesis.

The objective of this pilot study was to evaluate the feasibility of a randomized clinical trial to test whether short-term modifications in macronutrient intake could modify gene expression in prostate epithelium. We examined the effects of a low-fat/low-glycemic load diet in men with clinically localized prostate cancer who elected prostatectomy as their primary treatment choice. These results will help in the design of studies using gene expression or gene expression signatures as measures of the effect of dietary change on prostate cancer biology.

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Materials and Methods

Participants were men with clinically localized prostate cancer, who elected to undergo radical prostatectomy and received no neoadjuvant therapy. Study activities began within 2 weeks of diagnosis.

Eligibility criteria included the ability to implement dietary change, no concurrent disease requiring dietary modification, no current use of hormonal treatments, and body mass index >20 kg/m² and <35 kg/m². In addition, men had to have participated in an independent protocol that collected and stored four prostate tissue cores at the time of diagnostic prostate biopsy. Both the dietary intervention and biopsy tissue collection protocols were approved by the Institutional Review Boards of the Veterans Administration, Puget Sound Health Care System and the Fred Hutchinson Cancer Research Center, and all patients signed written informed consents. Study was completed between August 2003 and November 2004.

Randomization and Dietary Intervention. Participants were randomly assigned to one of two groups: group I received instructions to follow a low-fat/low-glycemic load diet ($<20\%$ energy from fat and total daily glycemic load <100) and group II was instructed to follow a "standard American" diet (35% energy from fat and total daily glycemic load >200). A detailed description of the intervention is available from the authors upon request. In brief, participants were provided a dietary intervention manual that focused on meal planning. For individuals randomized to the low-fat/low-glycemic load diet, the study nutritionist provided detailed instructions and worked with the participant to plan at least three meals. Participants randomized to the standard American group were asked to continue their usual dietary habits, using lists of prohibited and permitted foods as a guideline for food choices. During the first week, the nutritionist made up to five follow-up telephone calls to review the intervention materials and provide additional meal planning support; calls were made at least once per week thereafter.

Dietary and Anthropometric Assessment. Unscheduled, telephone-administered 24-h dietary recalls were completed each week to assess adherence to the study diet, using the University of Minnesota's Nutrition Data Systems for Research software (version 37, 2006). Interviewers were blinded to the randomization assignment. On the days of randomization and prostatectomy, participants were weighed by study staff on a beam balance scale. Height was measured using a standing stadiometer. Body mass index was calculated as weight (kg) / height (m)².

Clinical Specimens. At the time of biopsy and after acquiring 12 diagnostic prostate cores, we obtained four additional tissue cores from the peripheral zone. At the time of radical prostatectomy, the prostate was exposed via a midline incision, and we obtained four *in situ* prostate biopsy cores using an 18-gauge prostate needle biopsy gun (Bard Inc.). All biopsies were collected from the peripheral zone and immediately embedded and frozen as described below. Because our study was limited to the effects of diet on normal epithelium, we attempted to intraoperatively biopsy sites that did not

contain malignant epithelium, corresponding to sites from diagnostic biopsy specimens. The *in situ* samples were collected before removing the prostate because we previously found that surgical manipulation caused artifactual changes in gene expression (7).

Specimen Handling and Laser Capture Microdissection. Biopsy cores were embedded individually in polyethylene glycol freezing media (Tissue-Tek OCT Compound, Sakura Finetek), placed in isopentane that was precooled in liquid nitrogen, and stored at -80°C (8). We used laser capture microdissection to collect 5,000 epithelial cells from histologically benign epithelial glands and extracted RNA using a standardized protocol (9). The RNA was subjected to linear amplification and converted to cDNA for microarray hybridization as previously described (7).

Gene Expression Analysis by Microarray Hybridization. We prepared and hybridized spotted cDNA microarrays as previously described (10), using RNA from a single batch of reference standards for each hybridization. Fluorescent array images were collected for both Cy3 and Cy5 using a GenePix 4000B fluorescent scanner (Axon Instruments), and GenePix Pro 4.1 software was used to grid and extract image intensity data. Spots of poor quality, as determined by visual inspection, were removed from further analysis. To normalize log ratio data, a print tip-specific Lowess curve was fit to the log-intensity versus log-ratio plot, using 20.0% of the data to calculate the fit at each point. This curve was used to center the log-ratio for each spot. Data were filtered to exclude poorly hybridized cDNAs by removing values with average foreground minus background intensity levels less than 300. We used the average of the two duplicate cDNA spots on each microarray chip in subsequent analyses. Data were filtered to include clones returning data for at least 75% of the samples in both preintervention and postintervention groups, which reduced the initial list of 6,751 clones to 5,711 clones and 5,643 clones in the low-fat/low-glycemic load and the standard American diet groups, respectively.

Statistical and Pathway Analyses. We used the significant analysis of microarrays algorithm⁵ to analyze differences in transcript levels between preintervention and postintervention specimens (11). Paired, two-sample *t* tests were calculated for each transcript, and genes differentially expressed were identified using false discovery rates (FDR) ranging from 5% to 50%.

Quantitative Reverse Transcription-PCR. We used quantitative reverse transcription-PCR to validate microarray results for selected genes. Primers specific for the genes of interest were designed using the web-based primer design service Primer3 provided by the Whitehead Institute for Biomedical Research.⁶ We determined acceptable performance characteristics of the PCR primers using normal human prostate cDNA, Biolase Taq polymerase (Bioline Inc.), and the GeneAmp PCR system 9700 (Applied Biosystems) as previously described (8).

⁵ <http://www-stat.stanford.edu/~tibs/SAM/>

⁶ http://fokker.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi

Table 1. Postintervention dietary characteristics and intervention differences, by study arm

	Group I: low-fat/low-glycemic diet (n = 4)	Group II: standard American diet (n = 4)	P
	Mean (SD)	Mean (SD)	
Glycemic load	135 (6)	266 (37)	0.0004
Calories	1,466 (367)	2,394 (215)	0.004
Fat (g)	51 (36)	94 (8)	0.06
Carbohydrates (g)	178 (12)	309 (47)	0.002
Protein (g)	82 (11)	83 (13)	0.9
% Calories from protein	23 (3)	14 (3)	0.007
Fiber (g)	21 (4)	13 (4)	0.02
Preintervention weight (kg)	91.4 (20.1)	90.9 (12.8)	0.97
Postintervention weight change (kg)	-5.3 (1.7)	0.8 (4.5)	0.02*

*Test for difference in weight change between study groups, adjusted for baseline weight.

Total RNA from participant samples were reverse transcribed, and cDNA was purified as previously described. Relative quantification of gene expression by quantitative PCR (40 cycles of 60°C annealing, 72°C extension, and 95°C melting) was done on a 7700 Sequence Detector using SYBR Green Master mix and gene-specific primers following the manufacturer's recommendations.

Results

Participant Demographic and Clinical Characteristics. Median participant age was 64 years (range, 59-69), median body mass index was 29 (range, 23-35), median preoperative serum prostate-specific antigen was 5.2 ng/mL (range, 2.5-16.0), median prostate volume was 50 mL (range, 35-149), and all but one participant was Caucasian.

Intervention Effects on Diet and Weight. The intervention resulted in profoundly different dietary

patterns in the two study arms (Table 1). Compared with men in the standard American arm, participants in the low-fat/low-glycemic load arm consumed 39% less total energy, 46% less fat, 42% less carbohydrate, and 62% more fiber, and had a 51% lower glycemic load. The intervention effect for weight, defined as the difference in weight change between study arms adjusted for baseline weight, was 6.1 kg (95% confidence interval, 1.6-10.5 kg; $P = 0.02$).

Gene Expression Changes Associated with Dietary Intervention. Table 2 gives the distributions of gene expression differences in tissues obtained before and after dietary intervention. Differences in these tables are categorized symmetrically above and below zero in units of \log_2 . In both study arms, the relative difference in expression of ~95% of transcripts ranged between 0.67 and 1.49. In the standard American arm, expression of 20 transcripts decreased by 50% or greater, and 32 transcripts increased by 100% or greater. In the low-fat/low-glycemic load arm, expression of 9 transcripts decreased by 50% or greater, and expression of 43 transcripts

Table 2. Distribution of preintervention and postintervention differences in transcript expression, and number of significant differences at FDRs of 15%, 10%, and 5%, by treatment arm

	Preintervention vs. postintervention difference			Significant differences		
	Absolute difference (post/pre- \log_2)	Relative difference (post/pre- ratio)	Total, n (%)	FDR < 15% n (%)	FDR < 10% n (%)	FDR < 5% n (%)
Standard American arm (5,643 transcripts)	>+1.58	>3.0	3 (0.05)	0 (0)	0 (0)	0 (0)
	+(1.32-1.57)	2.50-2.99	5 (0.09)	0 (0)	0 (0)	0 (0)
	+(1.00-1.31)	2.00-2.49	24 (0.43)	0 (0)	0 (0)	0 (0)
	+(0.58-0.99)	1.50-1.99	180 (3.19)	0 (0)	0 (0)	0 (0)
	+(0.00-0.57)	1.00-1.49	2,549 (45.17)	0 (0)	0 (0)	0 (0)
	-(0.01-0.57)	0.67-0.99	2,658 (47.10)	0 (0)	0 (0)	0 (0)
	-(0.58-0.99)	0.50-0.66	204 (3.62)	0 (0)	0 (0)	0 (0)
	<-1.0	<0.50	20 (0.35)	0 (0)	0 (0)	0 (0)
Total			5,643 (100)	0 (0)	0 (0)	0 (0)
Low-fat/low-glycemic load arm (5,711 transcripts)	>+1.58	>3.0	11 (0.19)	11 (0.19)	10 (0.18)	6 (0.11)
	+(1.32-1.57)	2.50-2.99	8 (0.14)	5 (0.09)	4 (0.07)	0 (0)
	+(1.00-1.31)	2.00-2.49	24 (0.42)	3 (0.05)	2 (0.04)	0 (0)
	+(0.58-0.99)	1.50-1.99	118 (2.07)	1 (0.02)	0 (0)	0 (0)
	+(0.00-0.57)	1.00-1.49	2,595 (45.4)	0 (0)	0 (0)	0 (0)
	-(0.01-0.57)	0.67-0.99	2,831 (49.6)	0 (0)	0 (0)	0 (0)
	-(0.58-0.99)	0.50-0.66	115 (2.01)	3 (0.05)	3 (0.05)	3 (0.05)
	<-1.0	<0.50	9 (0.16)	7 (0.12)	7 (0.12)	7 (0.12)
Total			5,711 (100)	30 (0.53)	26 (0.46)	16 (0.28)

HUGO	NAME	PRE-DIET				POST-DIET				AVERAGE RELATIVE EXPRESSION
		1	2	3	4	1	2	3	4	
MMP7	Matrix metalloproteinase 7	Green	Green	Green	Green	Red	Red	Red	Red	13.1
OLFM4	Olfactomedin 4	Green	Green	Green	Green	Red	Grey	Red	Red	6.9
BF	B-factor properdin	Green	Green	Green	Green	Red	Red	Red	Red	6.4
IGF-2R	Insulin-like growth factor-2 receptor	Green	Green	Green	Green	Red	Red	Red	Red	3.5
VMP1	Likely ortholog of rat vacuole membrane protein 1	Green	Green	Green	Green	Red	Red	Red	Red	3.3
TGFB114	Transforming growth factor beta 1 induced transcript 4	Green	Green	Green	Green	Red	Red	Red	Red	3.2
IER3	Immediate early response 3	Green	Green	Green	Green	Red	Red	Red	Red	3.2
CCT2	Chaperonin containing TCP1, subunit 2 (beta)	Green	Green	Green	Green	Red	Red	Red	Red	3.1
HLA-DRB5	Major histocompatibility complex class II DR beta 4	Green	Green	Green	Green	Red	Red	Red	Red	3.1
CXCR4	Chemokine (C-X-C motif) receptor 4	Grey	Black	Green	Green	Red	Red	Red	Red	2.8
LUM	Lumican	Green	Green	Green	Green	Red	Red	Red	Red	2.8
CCL2	Chemokine (C-C motif) ligand 2	Black	Green	Green	Green	Red	Red	Red	Red	2.7
DUSP1	Dual specificity phosphatase 1	Green	Black	Green	Green	Red	Red	Red	Red	2.6
IER2	Immediate early response 2	Green	Green	Green	Green	Red	Red	Red	Red	2.5
SPARCL1	SPARC-like 1	Green	Black	Red	Green	Red	Red	Red	Red	2.3
TBX3	T-box 3	Green	Red	Green	Green	Red	Red	Red	Red	2.1
ETS1	V-ets erythroblastosis virus E26 oncogene homolog 1	Black	Green	Green	Green	Red	Red	Red	Red	2.0
SLC25A3	Solute carrier family 25 member 3	Red	Red	Red	Green	Red	Red	Red	Red	0.6
PRDX1	Peroxiredoxin 1	Red	Black	Red	Green	Red	Red	Red	Red	0.5
ABAT	4-aminobutyrate aminotransferase	Red	Red	Green	Black	Red	Red	Red	Red	0.4
FOLH1	Folate hydrolase (prostate-specific membrane antigen)	Red	Red	Green	Green	Red	Red	Red	Red	0.4
BPGM	23-bisphosphoglycerate mutase	Grey	Red	Red	Red	Red	Red	Red	Red	0.4
MGC15937	Similar to RIKEN cDNA 0610008P16 gene	Red	Red	Red	Red	Red	Red	Red	Red	0.3

Figure 1. Differential gene expression of pre-dietary versus post-dietary intervention (low-fat/low-glycemic load, group I) specimens. Paired two-sample *t* test results for presurgery versus postsurgery comparison. Twenty-six unique up-regulated genes with FDR ≤ 10% are listed. Average relative expression, change from preintervention to postintervention.

increased by 100% or greater. None of the differences in transcript expression in the standard American arm were statistically significant, even using a FDR as high as 50%. In contrast, in the low-fat/low-glycemic load arm, 30 (0.53%) of the cDNAs using a criterion of a 15% FDR and 16 (0.28%) of the cDNAs using a more conservative criterion of a 5% FDR were statistically different.

Figure 1 shows the list of 23 unique genes that showed significantly changed expression in the low-fat/low-glycemic load arm. Several genes are related to cell migration and tissue remodeling, including MMP7 (also called matrilysin), CXCR4, CXCL2, lumican, and SPARC-like 1. Others are involved in intracellular signal transduction, such as the immediate early response genes 2 and 3, the dual specificity phosphatase 1, and the v-ets oncogene homologue. Expression of insulin-like growth factor-II receptor transcripts increased, perhaps due to a positive feedback of the low-glycemic load diet. Genes that were down-regulated include prostate-specific membrane antigen and peroxiredoxin 1, which may play an antioxidant protective role in cells. We confirmed these microarray results by quantitative reverse transcription-PCR and found a correlation of 0.97.

Discussion

In this small randomized clinical trial, we showed the feasibility of studying dietary effects on gene expression using the preprostatectomy model. We collected both pretreatment biopsies and posttreatment *in situ* biopsies,

and delivered an effective dietary intervention within the weeks between diagnostic biopsy and surgery. We also showed that tissue from a single prostate biopsy core, weighing ~3 to 5 mg, yielded adequate RNA, albeit after a single round of amplification, for multiple analyses, including cDNA microarrays and quantitative reverse transcription-PCR. Lastly, we found that the intraindividual variability in gene expression in tissues collected at two time points is sufficiently small to allow the detection of intervention effects. Much larger sample sizes are needed to rigorously measure the reliability of transcript expression from prostate tissue collected at different times, but these results are encouraging for future research examining the effects of short-term interventions on prostate tissue.

The low-fat/low-glycemic load diet, and its associated weight loss, was associated with multiple gene expression changes. Many of these changes could conceivably alter the proliferation, metabolism, and redox potential of prostate epithelial cells. For example, insulin-like growth factor-I receptor binds both insulin-like growth factor-I and -II, and the role of insulin-like growth factor-I receptor in tumorigenesis and proliferation is well established (8). We emphasize, however, that much larger samples would be needed to make strong biological inferences based on gene expression arrays.

The principal limitation of this study is its small sample size. We had little power to detect modest changes in transcript expression. We also sampled only ~6,000 genes of the prostate tissue transcriptome, and many additional genes that were not evaluated could be

affected to an equal or greater extent. We also have not determined whether the diet-associated changes in transcript levels result in corresponding alterations in the cognate proteins. Lastly, we limited our analyses to the epithelial compartment, as prostate cancer arises from the epithelium, however, there are many interactions between the prostate stroma and epithelium (12-14).

In summary, we conclude that the effect of complex dietary changes on gene expression can be evaluated within the preprostatectomy model. These results provide important information for future studies that aim to examine the role of diet, obesity, and prostate carcinogenesis and/or progression.

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