Presurgical weight loss affects tumour traits and circulating biomarkers in men with prostate cancer

Wendy Demark-Wahnefried*[,1,2], Soroush Rais-Bahrami[2,3,4], Renee A Desmond[2,5], Jennifer B Gordetsky[3,6], Gary R Hunter[7], Eddy S Yang[2,8], Maria Azrad[1], Andrew D Frugé[1], Yuko Tsuruta[1], Lyse A Norian[1,2], Roanne Segal[9] and William E Grizzle[2,6]

[1]Department of Nutrition Sciences, Wallace Tumor Institute, University of Alabama at Birmingham, 1824 6th Avenue South, Suite 102, Birmingham, AL 35294, USA; [2]University of Alabama at Birmingham Comprehensive Cancer Center, Wallace Tumor Institute, 1824 6th Avenue South, Birmingham, AL 35294, USA; [3]Department of Urology, University of Alabama at Birmingham, 619 19th Street South, Birmingham, AL 35233, USA; [4]Department of Radiology, 619 19th Street South, Birmingham, AL 35233, USA; [5]Division of Preventive Medicine, University of Alabama at Birmingham, 1717 11th Avenue South, Birmingham, AL 35205, USA; [6]Division of Anatomic Pathology, Department of Pathology, University of Alabama at Birmingham, 619 19th Street South, Birmingham, AL 35233, USA; [7]Department of Human Studies, University of Alabama at Birmingham, 901 13th Street South, Birmingham, AL 35294, USA; [8]Department of Radiation Oncology, University of Alabama at Birmingham, 1700 6th Avenue South, Birmingham, AL 35233, USA and [9]Department of Medicine, University of Ottawa, 1053 Carling Avenue, Ottawa, ON K1Y 4E9, Canada

Background: Obesity is associated with aggressive prostate cancer. To explore whether weight loss favourably affects tumour biology and other outcomes, we undertook a presurgical trial among overweight and obese men with prostate cancer.

Methods: This single-blinded, two-arm randomised controlled trial explored outcomes of a presurgical weight loss intervention (WLI) that promoted ∼1 kg per week loss via caloric restriction and increased physical activity (PA). Forty overweight/obese men with clinically confirmed prostate cancer were randomised to the WLI presurgery or to a control arm; changes in weight, body composition, quality-of-life, circulating biomarkers, gene expression, and immunohistochemical markers in tumour and benign prostatic tissue were evaluated.

Results: The study period averaged 50 days. Mean (s.d.) change scores for the WLI vs control arms were as follows: weight: − 4.7 (3.1) kg vs − 2.2 (4.4) kg (P = 0.0508); caloric intake: − 500 (636) vs − 159 (600) kcal per day (P = 0.0034); PA: + 0.9 (3.1) vs + 1.7 (4.6) MET-hours per day (NS); vitality: + 5.3 (7.l4) vs − 1.8 (8.1) (P = 0.0491); testosterone: + 55.1 (86.0) vs − 48.3 (203.7) ng dl^{-1} (P = 0.0418); sex hormone-binding globulin: + 14.0 (14.6) vs + 1.8 (7.6) nmol l^{-1} (P = 0.0023); and leptin: − 2.16 (2.6) vs − 0.03 (3.75) (P = 0.0355). Follow-up Ki67 was significantly higher in WLI vs control arms; median (interquartile range): 5.0 (2.5,10.0) vs 0.0 (0.0,2.5) (P = 0.0061) and several genes were upregulated, for example, CTSL, GSK3B, MED12, and LAMC2.

Conclusions: Intentional weight loss shows mixed effects on circulating biomarkers, tumour gene expression, and proliferative markers. More study is needed before recommending weight loss, in particular rapid weight loss, among men with prostate cancer.

A recent consensus report indicates that obesity is a risk factor for 13 different cancers, with accumulating evidence that it may have a role in fatal prostate cancer (Lauby-Secretan et al, 2016). Contributing to this evidence is a multinational study of 10 106 prostate cancer cases

from 8 different cohorts, which found that for each 5-unit increase in body mass index (BMI: kg m^{-2}) pre-diagnosis, there was an 8% increase in prostate cancer specific mortality (P-trend = 0.01) (Yuan et al, 2015). A meta-analysis among 26 479 prostate cancer patients

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1303

found a 21% increased risk of biochemical recurrence with a similar increase in weight (relative risk: 1.21, 95% confidence interval: 1.11–1.31, $P < 0.01$) (Cao and Ma, 2011). Despite strong observational evidence that obesity is associated with more aggressive prostate cancer, few studies have explored whether intentional weight loss results in improved cancer control and/or possible mechanisms by which negative energy balance affects tumour biology and the host environment. Through such research, it may be possible to discover new supportive therapies and uncover signalling pathways that lead to the development of novel therapeutic agents (Hursting et al, 2012).

Limited research in breast cancer shows that weight loss interventions (WLIs) are safe for cancer survivors and improve health-related quality-of-life in the short-term (Reeves et al, 2014). A limited number of trials also suggest improvements in circulating inflammatory markers and adipokines, insulin, insulin-like growth factors, sex steroid hormones, and associated binding proteins (Reeves et al, 2014).

In prostate cancer only three randomised controlled trials (RCTs) of weight loss have been reported. RENEW (Reach Out to ENhancE Wellness in Older Cancer Survivors) is the largest and enrolled 261 prostate cancer survivors within a cohort that also included 380 breast and colorectal cancer survivors (Morey et al, 2009). In RENEW, significant reductions in body weight occurred and were associated with improved physical function (primary endpoint). As RENEW did not collect biospecimens, specific physiologic effects were not assessed. Two smaller weight loss trials ($n \leqslant 19$) (Schenk et al, 2009; Wright et al, 2013) were conducted in the presurgical setting. Here, significant weight loss was observed along with pre–post changes in serum insulin-like growth factor-binding protein-3 between the intervention and control arms; however, no differences were detected in insulin, C-peptide, IGF-1, and adiponectin (Wright et al, 2013). Neither of these trials assessed effects on tumour pathology.

Assessment of the impact of interventions directly on tumour tissue is a strength of presurgical trials—one that Kelloff et al (1994) initially proposed for testing chemopreventive agents. A key premise is that by monitoring intervention effects on Ki67, efficacy could be assessed more rapidly and with fewer participants (Kachroo and Gnanapragasam, 2013). The presurgical model also affords the opportunity to directly assess the impact of intervention on other biological mechanisms within the tumour. Presurgical models have been used to evaluate many therapeutic agents (Dowsett and Dunbier, 2008; Bonanni et al, 2012), but have rarely been used to assess the impact of lifestyle interventions. A phase II, RCT among 161 patients scheduled for prostatectomy, however, found significantly lower Ki67 proliferation rates in men randomised to a 3-week regimen of 30 g per day of ground flaxseed vs controls (Demark-Wahnefried et al, 2008), thus serving as proof-of-concept that non-pharmacologic interventions can be tested via presurgical trials. To date, there have been no presurgical trials that have assessed the impact of caloric restriction or increased physical activity (PA) on tumour biology. This report describes the results of a pioneering NCI-funded (R21 CA161263) feasibility trial that utilised a presurgical model to assess the effects of a WLI on tumour biology (Ki67 and gene expression), candidate biomarkers (e.g., circulating prostate specific antigen (PSA), androgens, growth hormones (vascular endothelial growth factor, VEGF), cytokines (tumour necrosis factor (TNF)-β), adipokines (leptin), insulin, and RNA gene expression), and quality-of-life in men with newly diagnosed prostate cancer, who elected radical prostatectomy.

MATERIALS AND METHODS

Methods of this presurgical RCT are published and can be readily accessed for details (Demark-Wahnefried et al, 2015). A brief summary follows.

Patients. Men aged $\geqslant 19$ years diagnosed with biopsy-confirmed prostate cancer, BMI of 25–50 kg m^{-2}, and scheduled for radical prostatectomy > 3 weeks from the point of contact at the University of Alabama at Birmingham (UAB) or the Urology Centers of Alabama were eligible. Men were excluded if they were unable to communicate in English or via telephone; had other active malignancies (exception: non-melanoma skin cancers) or health conditions that affected body weight, or precluded unsupervised PA (e.g., thyroid dysregulation, severe orthopedic conditions, or unstable/recent cardiovascular issues); had previous hormonal or neo-adjuvant treatment; were unwilling to be randomised; or were currently enrolled in a weight loss programme (note: exclusion was not based on PA level given the focus on short-term weight loss, for which caloric intake has a larger role (Jensen et al, 2014)).

Written informed consent was obtained from all interested and eligible men. The protocol was approved by the UAB Institutional Review Board and registered according to Consolidated Standards of Reporting Trials guidelines (NCT01886677). Recruitment began 7 December 2012 and the final participant completed the protocol 12 February 2015 and tumour specimens were released 1 year thereafter.

Study design. This two-arm, single-blinded, presurgical RCT was conducted among 40 overweight or obese men, newly diagnosed with prostate cancer. The experimental arm was assigned immediately to an energy-restricted diet plus aerobic PA to promote a weight loss of ~ 1 kg per week; the wait-list control arm was offered the intervention post surgery.

Objectives and assessments. Designed as a feasibility trial, the primary aims were attainment of the following benchmarks: (1) enrolment of 40 participants within 2 years; (2) $\geqslant 80\%$ retention; (3) adherence, defined as completion $\geqslant 70\%$ of contact sessions; and (4) safety, defined as the absence of serious adverse events in the intervention arm. Secondary (exploratory) aims included characterising pre–post between-arm differences on measures of body habitus and composition, energy intake, PA, physical functioning, quality-of-life, serum biomarkers, lymphocytic gene expression, and tumour markers. Phlebotomy and assessments were performed after a 12 h fast at baseline and follow-up (within 3 days before prostatectomy). Participants exhibiting uncontrolled Stage III hypertension or cardiac abnormality at baseline were cleared by their urologist before study continuation (Schmitz et al, 2010).

Measures/measurement points

Clinical measures. Anthropometric measures were conducted in light clothing, without shoes, and using standardised procedures (Lohman and Martorell, 1988). Body fat and lean mass were quantified via dual energy X-ray absorptiometry using a calibrated Lunar Prodigy densitometer (GE-Lunar Corporation, Madison, WI; software version 12.3). VO$_2$ assessments were performed using a standardised American College of Sports Medicine (ACSM) protocol in which workout intensity was increased in 4 min intervals to achieve 80% of maximum heart rate (MHR) (Kohl et al, 1990). Interval and total treadmill time was recorded. Protocol details are published in the methods paper (Demark-Wahnefried et al, 2015); the VO$_{2peak}$ equation is included in Supplementary Materials.

Patient-reported outcomes. Written surveys were administered at baseline and follow-up, to assess all prescribed and over-the-counter medications (including dietary supplements) and comorbidities (Older Americans Resources and Services Comorbidity Index) (Fillenbaum, 1988). Quality-of-life was assessed using the RAND-36, which generated summary scores for Physical Health

(from physical functioning, physical role limitations, pain, and general health subscales), and Mental Health (from emotional well-being, emotional role limitations, vitality, and social functioning subscales); quality-of-life related to urinary, bowel, and sexual functioning was captured using the Prostate Cancer Index) (Hays *et al*, 1993; Litwin *et al*, 1998). Physical Activity Recalls (7-Day) were administered by trained personnel (Blair *et al*, 1985). As indicated in the methods paper, (Demark-Wahnefried *et al*, 2015) objective PA data were captured using Polar RS400 heart rate monitors (Polar Electro, Inc., Lake Success, NY, USA) (equations are included in Supplementary Materials). Two-day dietary recalls were performed by registered dietitians at both time points (De Keyzer *et al*, 2011) and analysed for kilocalories, macronutrients, and diet quality using the NCI-developed ASA24 (Subar *et al*, 2012). All study participants were monitored semi-weekly for adverse events, although none occurred.

Circulating biomarkers. Blood was collected by venipuncture and separated into sera, plasma, neutrophils, leukocytes, and DNA, which were stored at $-80\,^{\circ}$C; RNA was dispersed in 0.5 ml of RNAlater and stored at $-20\,^{\circ}$C. All sera was batch-analysed in duplicate for leptin, insulin, glucose, total testosterone, sex hormone binding globulin (SHBG), PSA, TNF-β, and VEGF according to the manufacturer's directions. Details can be accessed in the methods paper, (Demark-Wahnefried *et al*, 2015) along with a specific description of gene expression profiling for phosphatase and tensin homologue, phosphatidylinositol 3 kinase (PI3K), MAPK, STAT, BCL2, and receptors for insulin, leptin, androgen (AR), VEGF (VEGFR), and TNF (FAS) on circulating RNA extracted from buffy coat.

Tumour biomarkers. Two pathologists, each blinded to study condition, assisted with this investigation. One reviewed clinical pathology reports and all slides for each case, selecting one slide and one block/case based on the presence of adequate tumour, and the representativeness of the specimen; the other confirmed tumour grade. Slides were prepared at a 1 : 100 dilution for Ki-67 (clone:SP6, Thermo Fisher Scientific, Pittsburg, PA, USA) and percent of positive cells were assessed. Slides for other tumour markers used a 1 : 20 dilution for 4E-BP1 (clone:11G12C11, Santa Cruz Biotechnology, Dallas, TX, USA), AR (clone: EPR1535(2), Abcam, Cambridge, MA, USA) and IR (Abcam); a 1 : 200 dilution for cleaved caspase-3 (Asp175, Cell Signaling Technology, Danvers, MA, USA); and a 1 : 2000 dilution for nuclear factor-κ-light-chain-enhancer of activated B (clone: F-6, Santa Cruz Biotechnology). Intensity levels (0,1,2,3) were multiplied by the percentage staining and divided by 100.

The NanoString nCounter system (Seattle, WA, USA) was utilised to explore genes involved with signalling and immune pathways within the tumour; this analysis was added *post hoc* (Demark-Wahnefried *et al*, 2015). Six cases from each study arm having adequate surgical specimen tumours were selected for analysis; cases from the intervention arm, who lost over the median amount of weight were matched on International Society of Urological Pathology grade, race, and age (± 5 years) to controls with stable weights. Tumour was identified and macrodissected from paraffin-embedded surgical specimens. RNA was isolated using RNeasy FFPE kits (Qiagen, Valencia, CA, USA). Samples were processed according to manufacturer's directions on the NanoString nCounter Flex system using the GX PanCancer Pathways Panel (770 genes representing 13 canonical cancer pathways: 606 pathway genes, 124 cancer driver genes, and 40 reference genes) and the GX PanCancer Immune Profiling Panel (770 genes for 24 immune cell types/populations, 30 common cancer antigens, and genes for overall immune response including key checkpoint blockade genes). All signals below the mean background plus 2 s.d. were considered below the limits of detection. A normalisation factor was calculated from the expression of well-characterised housekeeping genes and spiked-in exogenous positive controls in each sample and applied to raw counts from nCounterTM output. The Benjamini-Yekutieli (2001) false discovery rate correction at $P < 0.05$ was used, as genes are co-regulated within a handful of specific pathways.

Interventions. After baseline assessment, men were block-randomised on race (African American *vs* others) and baseline BMI (25–29.9 *vs* 30 +) to receive the WLI immediately or to a wait-list control arm that was offered the weight loss regimen after study completion (Jayachandran *et al*, 2009; Hadziabdic *et al*, 2015).

Weight loss intervention arm. The intervention followed American Heart Association, American College of Cardiology, and The Obesity Society guidelines for weight loss, which recommends a tripartite approach of caloric restriction, increased PA, and behaviour modification (Jensen *et al*, 2014). The Mifflin-St Jeor equation (Frankenfield *et al*, 2003) was used to calculate energy needs with subtraction of 1000 kcal per day to promote an average weight loss of $\sim$1 kg per week. Registered dietitians counselled participants on caloric goals and correcting nutrient deficiencies noted from 2-day dietary recalls via food sources. American Cancer Society dietary guidelines were followed (Rock *et al*, 2012). Dietary instructions were customised to patients' needs and preferences. Participants were provided with references and instructed to count calories or use exchange lists (American Diabetes Association 2007). Participants were instructed to weigh themselves daily; those not having a scale were provided one. Dietitian follow-up occurred semi-weekly with counselling provided face-to-face, via the telephone, or through email and in accordance with Social Cognitive Theory (Bandura, 2004).

Exercise physiologists provided instruction to incrementally and ultimately strive for an additional 250 kcal per day deficit through aerobic PA. Although a reduction in caloric intake is essential to weight loss regimens, guidelines suggest that regular PA also is beneficial to preserve lean mass and provide an additional energy sink to compensate for brief bouts of dietary non-adherence. Consistent with ACSM guidelines, each training session included a 5 min warm-up, the work-out, and then a cool-down of slow walking and stretching (Kohl *et al*, 1990). An incremental ramping of intensity and volume from 60 to 80% of MHR as per tolerance was employed. Given that few men obtained regular PA, starting workouts began at 10 min bouts and increased incrementally, ultimately striving for daily 30 min sessions. Participants were encouraged to exercise at UAB twice weekly on ergometers and treadmills, and five times per week at home. Heart rate monitors and instructions were provided to enhance and monitor adherence during home-based sessions. In cases where on-site exercise was not possible due to distance, regimens were adapted to semi-weekly telephone counselling and/or email exchanges based on downloading of heart rate monitor data.

Wait-listed control arm. Wait-listed men also were counseled weekly by dietitians on food sources to correct nutritional deficiencies identified from baseline 24 h recalls. Wait-listed men were offered a 6-week weight loss regimen post surgery.

Statistical considerations and analyses

Sample size and statistical power. Power calculations were based on weight loss data from older men (age 50 + years) enrolled in UAB weight loss programmes, that is, mean (s.d.) weight loss of 5.64 (3.13) kg over 10 weeks. An enrolment of 40 (20 per arm) with an assumption of 80% retention yields 98% power to detect a difference in means of 4.73 (Group 1 mean, μ_1 of -5.64 per Group 2 mean, μ_2 of -0.91) assuming equal variance and using a two-group, two-sided *t*-test ($\alpha = 0.05$).

Statistical comparisons. Between-arm differences at baseline were assessed by *t*-tests for continuous variables and Fisher's exact test or χ^2-test for categorical variables. Changes in anthropometric measures were computed by subtracting follow-up from baseline values. Between-arm changes were compared using generalised linear models with the change score serving as the dependent variable and the treatment group as a predictor, controlling for baseline measures of pretest scores. Similar models were used for dietary data. Leptin levels were not normally distributed and were log-transformed prior to analyses. Median baseline PSA levels were compared using Wilcoxon tests, as were Ki67 data; for non-zero Ki67 values of <5, a value of 2.5 was used. For other immunohistochemical markers comparisons between treatment and control groups at surgery were examined by *t*-tests. Regression models were used to assess relationships between changes in weight, body composition, and serum biomarkers with tumour proliferation rates. Statistical significance was predetermined at $\alpha = 0.05$. Nanostring data were analysed using the nSolver software; *P*-values and fold change values were calculated using nCounter default settings that take into consideration background signals measured in negative controls and a normalisation factor described above. Given the exploratory nature of this study, no adjustments were made for multiple comparisons.

RESULTS

This trial achieved 85% retention, 95% adherence, and documented no serious adverse events; the accrual target also was achieved but required six additional months (Demark-Wahnefried *et al*, 2015). The CONSORT diagram (Figure 1) shows that of the 101 men referred, 40 were enrolled and 34 completed the trial. Other than refusal, prostatectomy-related issues were leading reasons for ineligibility and cancellation of prostatectomy was the leading cause of attrition (four out of six dropouts). One patient withdrew consent upon notification of assignment to the control arm; another was discontinued upon subsequent determination of

metastatic disease. No differences were observed between enrollees *vs* non-enrollees or completers *vs* non-completers by age, race, or BMI.

Participant characteristics are provided in Table 1. The sample was comprised largely of middle-aged men who were representative of males in the southeastern US in terms of race and education (US Bureau of the Census, 2014). Most participants had moderately aggressive prostate cancer and reported at least three other comorbidities, with roughly half reporting cardiovascular disease and a quarter reporting diabetes. The sample was evenly divided between overweight and obese men. The average length of time on study was 50 days. No statistically significant differences were detected between study arms regarding any of these factors.

Table 2 documents baseline-to-follow-up differences by study arm in body weight and composition, dietary intake, PA, function and fitness, and quality-of-life. Both study arms lost appreciable amounts of weight over the brief study period, with a weight loss of 5% noted in the WLI arm and 2.2% in the control arm. Between-arm differences in weight and BMI over the study period reached borderline significance. Lean and fat mass decreased in both groups with no between-arm differences detected. A significant correlation was observed between the rate of weight loss and loss in lean mass, $\rho = 0.632$ ($P = 0.0001$).

Although baseline caloric intakes were lower than expected, possibly due to increased stress with a recent diagnosis, under-reporting, or heightened awareness of dietary intake occurring with enrolment in a weight loss trial, caloric intake decreased significantly more over the study period in the WLI arm compared with controls. Significant reductions in intakes across all major macronutrient groups were also observed; however, the relative proportion of carbohydrate, protein, and fat remained stable and did not differ between arms. Slight decreases over time in diet quality were noted in both groups – an effect attributed to decreased dietary variety concomitant with decreased overall intakes.

Both arms also reported modest increases in weekly minutes of moderate-to-vigorous PA, although no between-arm differences were observed and the net impact on weekly Metabolic Equivalent
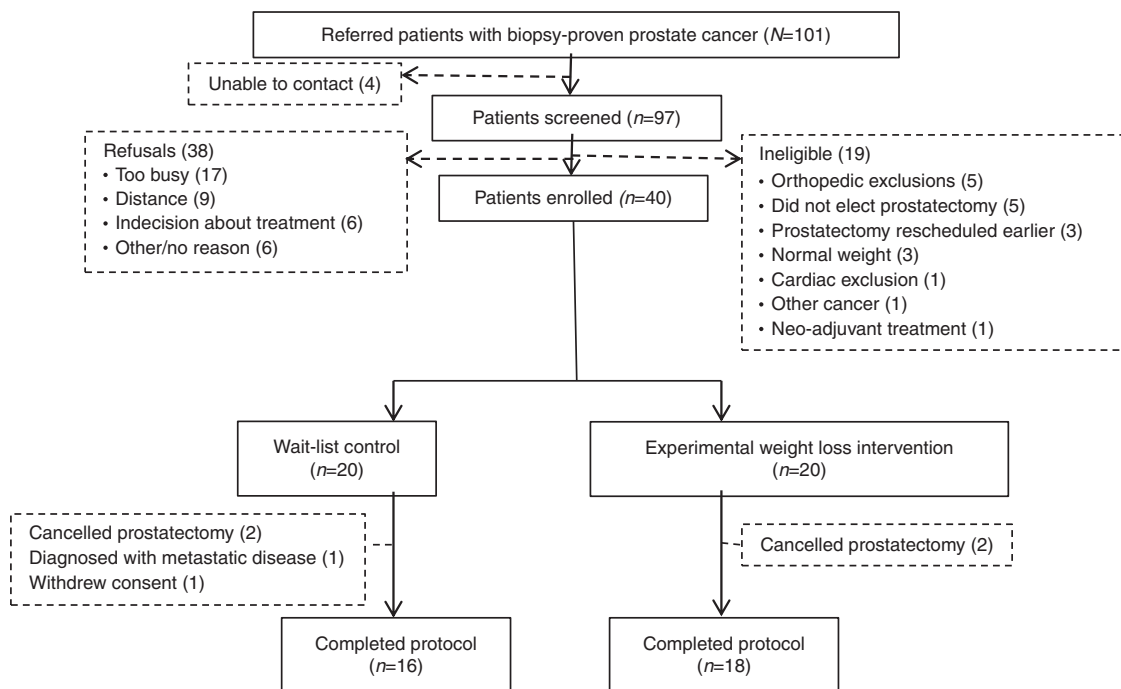

Figure 1. CONSORT diagram.

Table 1. Baseline characteristics of participants

Characteristic	Total (n = 40)	Wait-list control (n = 20)	Weight loss (n = 20)
Age (years)			
Mean (s.d.)	60.1 (6.3)	60.2 (5.9)	59.9 (6.9)
Range	46–73	51–73	46–72
Race (n (%))			
African American	12 (30)	6 (30)	6 (30)
Non-Hispanic White	28 (70)	14 (70)	14 (70)
Education (n (%))			
High school graduate	8 (20)	5 (25)	3 (15)
Some college/technical	12 (30)	5 (25)	7 (35)
College graduate	8 (20)	4 (20)	4 (20)
Post graduate	12 (30)	6 (30)	6 (30)
Comorbidities (n (%))			
0	3 (7.9)	1 (5.3)	2 (10.5)
1–2	12 (31.6)	5 (26.3)	7 (36.8)
3+	23 (60.5)	13 (64.4)	10 (52.7)
Cardiovascular disease (n (%))	21 (53.9)	13 (68.4)	8 (40.0)
Diabetes (n (%))	10 (25)	4 (20)	6 (30)
Current smoker (n (%))	5 (12.5)	2 (10)	3 (15)
International Society of Urological Pathology prostate cancer grade of biopsy (n (%))			
Grade Group 1 (Gleason sum ⩽6)	11 (27.5)	5 (25.0)	6 (30.0)
Grade Group 2 (3,4)	16 (40.0)	7 (35.0)	9 (45.0)
Grade Group 3 (4,3)	8 (20.0)	5 (25.0)	3 (15.0)
Grade Group 4 (Gleason sum 8)	5 (12.5)	3 (15.0)	2 (10.0)
Body mass index			
Mean (s.d.)	31.4 (4.5)	31.8 (5.1)	30.9 (3.9)
Range	25.2–45.8	25.4–45.8	25.2–40.3
Overweight (n (%))	18 (45)	8 (40)	10 (50)
Obese (n (%))	22 (55)	12 (60)	10 (50)
Days on protocol			
Mean (s.d.)	49.6 (23.1)	52.2 (24.0)	47.0 (22.6)
Range	17–95	17–94	20–95

Hours of PA was negligible. Objectively measured PA showed slight decreases in both groups. However, no between-arm differences were seen for either of these measures or for fitness.

Little change was noted over time in most physical or emotional quality-of-life subscales or summary scores. However, compared with controls, the WLI arm reported significant improvements in vitality and erection frequency.

Significant between-arm differences were noted in total testosterone and SHBG, with both biomarkers showing strong increases in the WLI arm as compared with controls (Table 3). These concomitant increases of the hormone and its binding protein balanced out the net effect on free androgen index, which remained stable. The WLI arm also experienced significant reductions in leptin as compared with controls. No other between-arm differences were observed with respect to other circulating biomarkers. There also were no significant between-arm differences in corresponding gene expression within the buffy coat.

Sufficient tissue was secured on 68% of biopsies and 100% of surgical specimens. Table 4 chronicles the immunohistochemical results in both the tumour and benign tissue, and within biopsy and surgical specimens. Compared with controls, the WLI arm manifested significantly greater Ki67 proliferation rates at the time of surgery. Change in tumour Ki67 from biopsy to surgery for individual participants is plotted in Figure 2 by study arm, with data provided in Supplementary Table 1. No other between-arm differences were noted in other tumour markers in either the benign or malignant tissue. No significant associations were found between circulating levels of testosterone, SHBG, or leptin, and proliferation rates in the tumour.

The heat map for the top 20 genes corresponding to the signalling and immune pathways that distinguished tumours from the WLI vs control arms shows significant between-arm differences in 13 genes related to signalling and 8 genes related to immune function (Figure 3). The signalling panel detected significant downregulation of serine/threonine kinase 2 (35% reduction; $P = 0.0375$) and Ephrin A5 (EFNA5; 48% reduction; $P = 0.0098$), and upregulation of the following genes: AT-rich interaction domain 1A (ARID1A; 14% increase; $P = 0.0330$); mediator of DNA damage checkpoint 1 (MDC1; 14% increase; $P = 0.0078$); SWI/SNF-related matrix-associated actin-dependent regulator of chromatin (SMARCA4; 19% increase; $P = 0.0276$); PHD finger protein 6 (PHF6; 22% increase; $P = 0.0199$); glycogen synthase kinase 3β (GSK3B; 23% increase; $P = 0.0144$); mediator of RNA polymerase II transcription subunit 12 (MED12; 27% increase; $P = 0.04547$); BCL6 corepressor (BCOR; 35% increase; $P = 0.0360$); activin A receptor 1B (ACVR1B; 36% increase; $P = 0.0475$); Cal proto-oncogene C (CBLC; 51% increase; $P = 0.0290$); DNA polymerase β (POLB; 61% increase; $P = 0.0255$); and Laminin subunit γ-2 (LAMC2; 207% increase; $P = 0.0480$). In addition, the immune panel detected significant downregulation of musculoaponeurotic fibrosarcoma (MAF; 35% decrease; $P = 0.0347$) and upregulation of the following genes: signal transducer and activator of transcription 5 (STAT 5B; 14% increase; $P = 0.0403$); activating transcription factor 1 (ATF1; 23% increase; $P = 0.0168$); Cathepsin L (CTSL; 29% increase; $P = 0.0497$); transcription factor EB (TFEB; 39% increase; $P = 0.0403$); histocompatibility complex, class II, DP Beta 1 (HLA-DPB1; 40% increase; $P = 0.0305$); mannose receptor C type 1 (MRC1; 40% increase; $P = 0.0134$); and cluster of differentiation 86 (CD86; 66% increase; $P = 0.0283$).

DISCUSSION

This trial is the first to document the effects of a presurgical WLI on tumour proliferation rates and other outcomes in patients with cancer. However, instead of reduced tumour proliferation rates as hypothesised, greater proliferation rates were observed with the WLI. As this is the first weight loss trial in the presurgical setting, it is difficult to draw comparisons with other studies, although we had forecasted effects similar to Fabian et al (2013). In that single-arm weight loss study, a median weight loss of 11% was observed over a 6-month period and Ki67 decreased from 1.4 to 0.4% ($P = 0.041$) in the subset of women who had detectable proliferation rates in fine-needle periareolar aspirates. However, the Fabian trial differed from the current trial. First, it was performed in women and specifically in healthy women without cancer. Second, the rate of weight loss (%body weight loss/day) was 64% faster in the current presurgical trial. Given that Ki67 is expressed more in malignant vs benign tissue and given the steeper trajectory of weight loss in the current trial, we expected an accentuated effect, not one in the opposite direction. This calls into question whether negative energy balance exerts differential effects in transformed cells compared with normal cells. Support for this premise is provided by our data that show no between-arm differences in benign tissue.

Our findings in malignant tissue correspond more to those of Kristal et al (2005), who found increases in Ki67 at 18-month follow-up in the weight loss arm of an RCT of 87 Barrett's oesophagus patients compared with reduced rates among controls. Our findings also parallel those of a recent presurgical trial of metformin among 200 breast cancer patients by Bonanni et al

Table 2. Baseline to follow-up change in weight status, body composition, diet, physical activity, fitness and quality-of-life

	Wait-list control			Weight loss intervention			
	Baseline (n = 16)	Follow-up (n = 16)		Baseline (n = 18)	Follow-up (n = 18)		
	Mean (s.d.)	Mean (s.d.)	Change (s.d.)	Mean (s.d.)	Mean(s.d.)	Change (s.d.)	P-value
Weight (kg)	96.1 (13.6)	93.9 (11.9)	− 2.2 (4.4)	94.7 (12.5)	90.0 (12.7)	− 4.7 (3.1)	0.0508
BMI (kg m^{-2})	30.4 (3.3)	29.7 (2.4)	− 0.8 (1.4)	30.8 (3.9)	29.3 (3.9)	− 1.5 (1.0)	0.0677
Body fat (kg)	33.64 (8.97)	31.77 (7.73)	− 1.87 (3.25)	32.18 (8.19)	29.06 (7.93)	− 3.12 (2.19)	0.1354
Body fat (%)	35.8 (4.8)	34.8 (4.4)	− 1.0 (2.0)	35.1 (5.3)	33.2 (5.2)	− 1.9 (1.7)	0.1691
Android body fat (%)	45.5 (5.9)	44.1 (6.6)	− 1.4 (3.0)	44.8 (7.5)	41.7 (7.4)	− 3.1 (3.6)	0.1404
Gynoid body fat (%)	33.1 (5.4)	32.3 (4.6)	− 0.8 (2.2)	30.9 (5.1)	32.4 (5.1)	− 1. 5 (2.3)	0.3034
Lean body mass (kg)	58.76 (5.94)	58.37 (5.15)	− 0.39 (1.78)	58.44 (0.64)	57.24 (6.21)	− 1.19 (1.02)	0.0903
Caloric intake (kcal per day)	1702 (526)	1543 (431)	− 159.2 (600)	1551 (542)	1039 (392)	− 500 (636)	**0.0034**
Dietary fat intake (g)	81 (25)	69 (24)	− 12.1 (32.7)	65 (25)	44 (24)	− 21.1 (33.4)	**0.0151**
Dietary carbohydrate intake (g)	160 (77)	151 (56)	− 9.3 (77.0)	168 (67)	113 (46)	− 53.8 (60.7)	**0.0254**
Dietary protein intake (g)	77 (28)	67 (28)	− 10.5 (31.0)	68 (23)	46 (16)	− 21.7 (28.2)	**0.0316**
Macronutrient distribution (% of kcal)							
Carbohydrate	37.4 (11.4)	40.5 (11.6)	3.2 (10.5)	42.7 (7.4)	44.0 (10.3)	1.3 (10.3)	0.8993
Total fat	43.2 (5.5)	39.9 (7.5)	− 3.3 (10.5)	37.5 (5.3)	36.5 (10.7)	− 1.1 (10.0)	0.5094
Protein	18.3 (4.5)	17.4 (4.5)	− 0.9 (3.70)	18.4 (4.5)	18.9 (6.2)	0.51 (6.2)	0.3958
Healthy eating index#	64.9 (9.2)	61.1 (8.4)	− 3.8 (10.3)	66.3 (8.6)	63.6 (7.8)	− 2.7 (7.6)	0.4629
Self-reported PA							
Min./week of vigorous PA	6.6 (14.7)	26.2 (42.3)	19.7 (43.6)	34.3 (70.3)	63.4 (71.4)	29.2 (79.6)	0.7733
Min./week of moderate PA	70.0 (77.3)	94.1 (104.0)	24.1 (73.0)	55.4 (73.4)	67.1 (74.7)	11.7 (84.2)	0.6596
MET-hours per day	30.5 (2.6)	33.2 (5.6)	1.7 (4.6)	31.6 (3.7)	32.5 (3.5)	0.9 (3.1)	0.5396
Heart rate assessed PA							
MET-hours per day	31.0 (3.7)	29.1 (3.4)	− 1.8 (5.5)	31.4 (4.4)	28.4 (3.7)	− 3.0 (4.4)	0.6355
Fitness							
V0$_{2\ peak}$ (ml O$_2$ per kg min^{-1})	24.9 (4.2)	26.5 (2.3)	1.6 (3.7)	24.5 (3.8)	24.2 (5.1)	− 0.3 (3.9)	0.1770
Heart rate treadmill 2 MPH (beats per min)	90.3 (10.5)	86.1 (9.5)	− 4.2 (7.8)	100.6 (13.0)	93.8 (16.2)	− 6.8 (11.2)	0.4668
Health-related quality of life							
Physical health summary score	86.1 (11.0)	87.1 (15.2)	1.1 (15.9)	78.9 (19.4)	78.9 (22.3)	− 0.04 (11.0)	0.6793
Physical functioning	91.0 (8.7)	91.7 (11.9)	0.7 (11.0)	82.8 (20.1)	90.6 (17.3)	7.8 (22.0)	0.7612
Physical role limitations	86.7 (31.1)	85.0 (35.1)	− 1.7 (50.4)	85.9 (30.2)	81.3 (40.3)	− 4.7 (27.7)	0.7934
Pain	92.2 (10.5)	92.8 (19.7)	0.7 (15.0)	80.3 (24.5)	77.7 (26.4)	− 2.7 (7.6)	0.5780
General health	74.4 (15.0)	79.0 (9.1)	4.6 (9.4)	66.6 (21.5)	65.9 (19.7)	− 0.6 (18.4)	0.0562
Emotional health summary score	85.8 (7.7)	82.9 (12.4)	− 2.9 (8.4)	81.0 (13.0)	81.4 (15.1)	0.4 (8.0)	0.2580
Emotional well-being	92.5 (7.3)	91.7 (6.5)	− 0.8 (7.6)	84.8 (12.6)	87.3 (12.2)	2.5 (8.5)	0.8484
Emotional role limitations	93.3 (18.7)	86.7 (30.3)	− 6.7 (28.7)	95.8 (16.7)	89.6 (29.1)	− 6.3 (25.0)	0.9170
Vitality	61.8 (10.3)	60.0 (12.4)	− 1.8 (8.1)	52.8 (16.3)	58.2 (15.8)	5.3 (7.4)	**0.0491**
Social functioning	95.8 (7.7)	93.3 (12.4)	− 2.5 (10.8)	90.6 (17.4)	90.6 (17.4)	0 (19.4)	0.9417
Other functioning							
Urinary function	2.07 (1.14)	1.69 (0.85)	− 0.31 (1.25)	1.36 (0.75)	1.36 (0.75)	0.00 (0.39)	0.7865
Bowel function	4.21 (1.47)	4.57 (0.93)	0.36 (0.63)	4.64 (0.74)	4.86 (0.36)	0.21 (0.69)	0.6682
Sexual function	3.28 (1.38)	3.43 (1.02)	0.14 (0.77)	2.93 (1.21)	3.14 (1.35)	0.21 (0.69)	0.9604
Erection quality	3.79 (0.43)	3.72 (0.61)	− 0.07 (0.62)	3.00 (1.17)	3.07 (1.21)	0.07 (1.07)	0.6114
Erection frequency	4.29 (1.07)	3.77 (1.24)	− 0.46 (0.66)	3.14 (1.70)	3.36 (1.55)	0.21 (0.43)	**0.0182**

Abbreviations: BMI = body mass index; PA = physical activity. Note: n = 15 control, n = 16 weight loss for weight data, quality of life data; n = 15 control, n = 15 weight loss for DXA data; n = 16 control, n = 17 weight loss for HEI index; n = 16 self-reported PA both arms; n = 10 control, n = 9 weight loss for HR-assessed PA; n = 16 control, n = 15 weight loss for fitness; n = 14 epic data both arms; n = 16 ASA survey weight loss. Bold values are statistical significance of P values.

(2012) who observed nonsignificant increases in Ki67 in the metformin arm, who experienced modest weight loss.

Tumour gene expression data, however, support some of our expectations. For example, we found tumour *MAF* downregulation in WLI men; thus, corroborating findings of Sharad *et al* (2011) who reported MAF upregulation in tumours of men with high *vs* low BMIs. Likewise, we expected and found downregulation of *EFNA5*, a gene associated with insulin secretion; however, we did not see between-arm differences in circulating insulin levels, likely due to the pharmacologic treatment of roughly one-fourth of our sample, who reported a diagnosis of diabetes.

As obesity is associated with decreased immune status (Valdes-Ramos and Benitez-Arciniega, 2007), we hypothesised upregulation of genes associated with immune response among men who lost weight. Our data support this premise, as *MRC1*, *HLA-PB1*, and *CD86* are all indicators of dendritic cell maturation, the cells most able to stimulate T-cell immunity (Karthaus *et al*, 2012). These data also provide an alternate explanation for the elevated

Table 3. Baseline to follow-up change in circulating biomarkers

	Wait-list control			Weight loss intervention			
	Baseline (n = 16)	Follow-up (n = 16)		Baseline (n = 18)	Follow-up (n = 18)		
	Mean (s.d.)	Mean (s.d.)	Change (s.d.)	Mean (s.d.)	Mean(s.d.)	Change (s.d.)	P-value
Testosterone ng dl^{-1}	369.1 (192.9)	320.8 (119.6)	− 48.3 (203.7)	366.4 (222.5)	421.5 (231.8)	55.1 (86.0)	**0.0418**
SHBG nmol l^{-1}	45.2 (17.2)	46.9 (14.7)	1.8 (7.6)	55.6 (50.3)	69.5 (47.9)	14.0 (14.6)	**0.0023**
Free androgen index	32.7 (26.5)	24.2 (7.3)	− 8.6 (26.8)	25.6 (8.9)	23.0 (8.5)	− 2.6 (7.6)	0.8562
VEGF pg ml^{-1}	122.6 (67.6)	117.5 (78.1)	− 5.14 (36.8)	107.3 (71.5)	105.4 (74.2)	− 1.9 (59.0)	0.9587
TNF-β pg ml^{-1}	0.25 (0.06)	0.27 (0.07)	0.03 (0.05)	0.28 (0.09)	0.31 (0.13)	0.04 (0.14)	0.7818
Insulin μU ml^{-1}	14.8 (11.7)	17.2 (25.2)	2.3 (22.5)	11.8 (6.5)	10.2 (5.7)	− 1.63 (4.92)	0.4841
Glucose mg dl^{-1}	109.8 (14.6)	110.2 (16.2)	0.4 (21.6)	113.5 (19.2)	113.6 (17.7)	0.14 (10.4)	0.8070
HOMA	2.0(1.4)	1.6 (0.8)	− 0.4 (1.2)	1.7 (0.8)	1.4 (0.8)	− 0.2 (0.7)	0.9878
Leptin ng ml^{-1}	15.0 (6.2)	15.0 (6.7)	− 0.03 (3.75)	14.9 (7.7)	12.7 (8.4)	− 2.16 (2.6)	**0.0355**
PSA (median) ng dl^{-1}	5.45	4.95		5.30	5.40		0.655
	Gene expression of receptors, transcription factors and regulators of apoptosis						
	Relative expression from baseline (n = 12)			Relative expression from baseline (n = 12)			
AR	1.542 (0.4316)			2.805 (0.8323)			0.1915
VEGFR	1.775 (0.7461)			1.805 (0.4972)			0.9744
TNF receptor (FAS)	1.070 (0.2530)			1.444 (0.3089)			0.3589
INSR	1.303 (0.2921)			1.523 (0.3730)			0.6462
LEPR	1.073 (0.1980)			1.523 (0.2484)			0.1705
FLT1	1.515 (0.4815)			1.361 (0.3429)			0.7964
MAPK	1.191 (0.1993)			1.381 (0.2226)			0.5307
STAT	0.999 (0.1977)			1.123 (0.1419)			0.6150
BCL2	1.074 (0.2725)			1.513 (0.3349)			0.3194

Abbreviations: AR = androgen receptor; INSR = insulin receptor; LEPR = leptin receptor; MAPK = mitogen-activated protein kinase; PSA = prostate specific antigen; SHBG = sex hormone binding globulin; STAT = signal transducer and activator of transcription; TNF-β = tumour necrosis factor-β; VEGF = vascular endothelial growth factor; VEGFR = VEGF receptor. Bold values are statistical significance of P values. Note: N = 16 weight loss testosterone, SHBG, free androgen index.

Ki67 detected within tumours in the WLI group: increased intra-tumoural immune activation following weight loss could contribute to the cellular proliferation detected by Ki67 staining. This hypothesis also is consonant with evidence that weight loss liberates free fatty acids from adipose stores and can trigger inflammation (Calder, 2015), whereas the influx of free fatty acids is generally transitory and the body quickly utilises them for fuel, in the case of more rapid weight loss and little increase in PA to 'burn-off' the residual, there may be greater potential for inflammation.

We also hypothesised the upregulation of DNA repair and indeed the increased gene expression of POLB and MDC1 in the WLI arm bear this out (Wang et al, 2015). A functional co-activator of AR, MDC1 influences cis-regulatory activity of AR target genes via histone H3 acetylation and, when knocked down, has been shown to increase the growth and migration of both CWR22Rv1 and LNCaP cells (Wang et al, 2015). Thus, within tumours of men losing more weight, we found evidence of favourable immune and insulin regulation, as well as DNA repair.

In contrast, our results also showed upregulation of genes associated with increased transcription, proliferation, migration, and invasion, for example, STAT5B, ATF1, TFEB, PHF6, ACVR1B, MED12, GSK3B, and LAMC2, with increases ranging from 14% (STAT5B) to 207% (LAMC2). GSK3B, MED12, and LAMC2 have been suggested as therapeutic targets because of their association with the Wnt signalling/B-catenin pathway, effects on AR, PI3K, and AKT, and contribution to more aggressive prostate cancer (Edwards et al, 2003; Salas et al, 2004; Barbieri et al, 2012).

Therefore, their upregulation with weight loss is unsettling, but may explain the increased Ki67 observed in the WLI vs control arms. Another upregulated gene of interest is ACVR1B given its association with androgens, immunosuppression, and carcinogenesis (Nomura et al, 2013).

In men assigned to the WLI, CTSL also was upregulated. This gene is associated with protein catabolism and may be driven by losses in lean mass concomitant with weight loss (Parr et al, 2013). Similar to most weight loss trials, we also detected a solid correlation between the rate of weight loss and the loss of lean mass, although it is interesting to see that weight loss invokes similar catabolic gene expression profiles within the prostatic tumour, as observed in heart or skeletal muscle. Upregulation of CTSL is of concern because of its association with increased osteoclast formation, bone resorption, bone loss, and metastatic potential (Sudhan et al, 2016). Given that the intervention was effective in reducing caloric intake, but not as effective in increasing PA, it is speculated that perhaps the losses in lean mass could have been mitigated and the upregulation of CTSL diminished if we had been able to activate men in the WLI arm, and include both aerobic and resistance PA.

Regarding the host environment, it is clear that the WLI significantly increased both total testosterone and its binding protein (SHBG), thus corroborating results of previous studies (Allan and McLachlan, 2010). Despite significant changes in circulating biomarkers, none of these differences correlated with tumour Ki67. However, the between-arm differences in erection frequency could be the result of androgen shifts. In addition to sex

Table 4. Immunohistochemical results on tumour and benign tissue in biopsy and surgical specimens

	Tumour tissue			
	Wait-list control		Weight loss intervention	
	Biopsy ($n = 9$)	Surgery ($n = 16$)	Biopsy ($n = 14$)	Surgery ($n = 18$)
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Androgen receptor	0.8 (0.6, 1.6)	1.7 (1.0, 2.0)	1.5 (0.1, 2.7)	1.8 (0.8, 2.85)
Insulin receptor	2.0 (1.4, 2.0)	1.0 (1.0, 2.0)	2.0 (2.0, 3.0)	1.9 (1.0, 2.0)
NF-κB p65	1.7 (1.1, 1.9)	1.0 (0.95, 1.8)	1.9 (1.0, 2.0)	1.2 (1.0, 1.9)
4E-BP1	0.45 (0.13, 1.10)	0.85 (0.45, 1.2)	0.6 (0.1, 1.2)	1.3 (0.6, 1.7)
Ki67 (%)	2.5 (2.5, 10.0)	0.0 (0.0, 2.5)	2.25 (0.0, 5.0)	5.0 (2.5, 10.0)[a]
	Benign tissue			
	Wait-list control		Weight loss intervention	
	Biopsy ($n = 13$)	Surgery ($n = 16$)	Biopsy ($n = 17$)	Surgery ($n = 18$)
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Androgen receptor	0.6 (0.2, 1.2)	1.6 (0.5, 1.8)	0.8 (0.4, 1.8)	1.7 (1.0, 2.25)
Insulin receptor	2.0 (2.0, 2.0)	2.0 (1.7, 2.0)	2.0 (2.0, 2.0)	2.0 (1.8, 2.0)
NF-κB p65	2.0 (0.4, 1.8)	1.6 (0.95, 2.0)	2.0 (2.0, 2.0)	1.6 (0.9, 1.8)
4E-BP1	0.0 (0.0, 0.05)	0.15 (0.08, 0.3)	0.0 (0.0. 0.05)	0.1 (0.1, 0.2)
Ki67 (%)	0.0 (0.0, 0.0)	0.0 (0.0, 3.75)	0.0 (0.0, 2.5)	2.5 (0.0, 5.0)

Abbreviations: IQR = interquartile range; NF-κB = nuclear factor-κB.
[a]Ki67 (%) difference between group at surgery Wilcoxon's rank-sum test $P = 0.0061$.
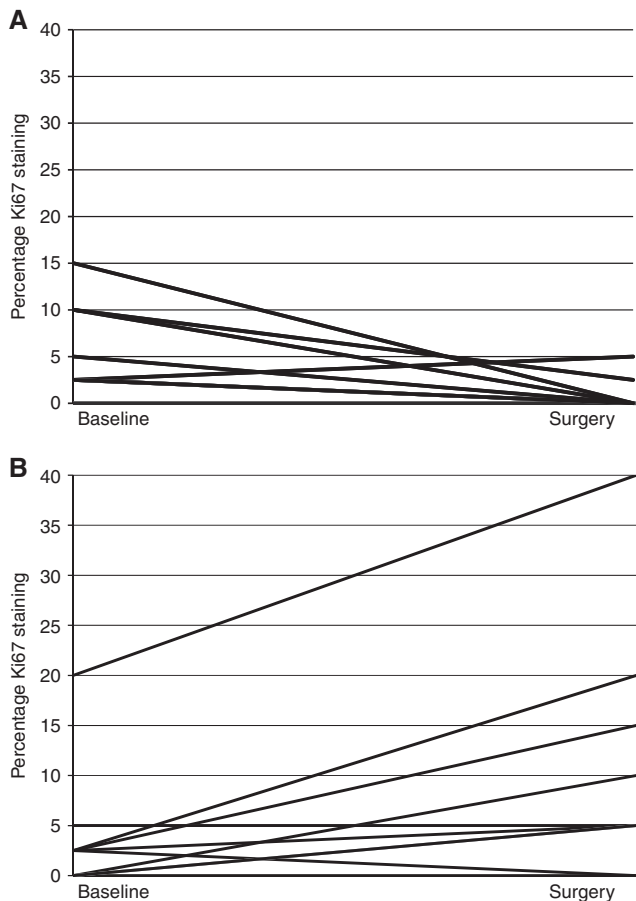

A

B

Figure 2. Tumour Ki67 for controls (A) and weight loss group (B) at biopsy and surgery ($P = 0.0298$ controls baseline-to-surgery; $P = 0.0128$ weight loss group baseline-to-surgery).

hormone differences, we, similar to others (Rosenbaum and Leibel, 2014), observed significant decreases in circulating leptin in the WLI *vs* control arms.

Importantly, significant improvements in vitality were reported by WLI men as compared with controls. Similar between-arm differences were observed in the previously described RENEW trial (Morey *et al*, 2009). Although other differences in quality-of-life domains may exist, the large variation in response coupled with the small sample size likely precluded our ability to detect differences. Moreover, although the intervention was successful in promoting caloric restriction, it did not result in significant increases in PA, which may have increased quality-of-life to a greater extent (Buffart *et al*, 2017).

This trial had other limitations besides sample size. Multiple testing increases the risk for false positive findings. Although some correction was used to protect against false discovery in gene expression, this risk still remains for these and other outcomes. Thus, future research is needed to confirm findings of this hypothesis-generating, feasibility trial. A limitation uncommon in pharmacologic trials, but concerning in behavioural interventions, especially among motivated, recently diagnosed patients with cancer, is that of 'drop-in' (Steins Bisschop *et al*, 2015). Drop-in was a significant issue in this trial, as men in the control group reported caloric reductions, more PA, and lost weight; this drop-in likely attenuated between-arm differences in these factors and others. Prostate cancer also imposes additional limitations: (1) diagnosis from small foci of disease, thus limiting tissue available for pre-post comparisons (adequate tissue was only available on 68% of biopsy specimens); (2) multi-focality, thus making pre-post comparisons problematic (hence we emphasise between-arm comparisons of surgical specimens, Table 4); and (3) low proliferation rates, increasing the risk of artifact and attenuating the ability to observe differences. Strengths of this presurgical trial are that it is the first ever to assess effects of intentional weight loss on tumours in humans, differences in Ki67 and other outcomes were detected, and the trial met most feasibility benchmarks, that is, retention, adherence, and safety
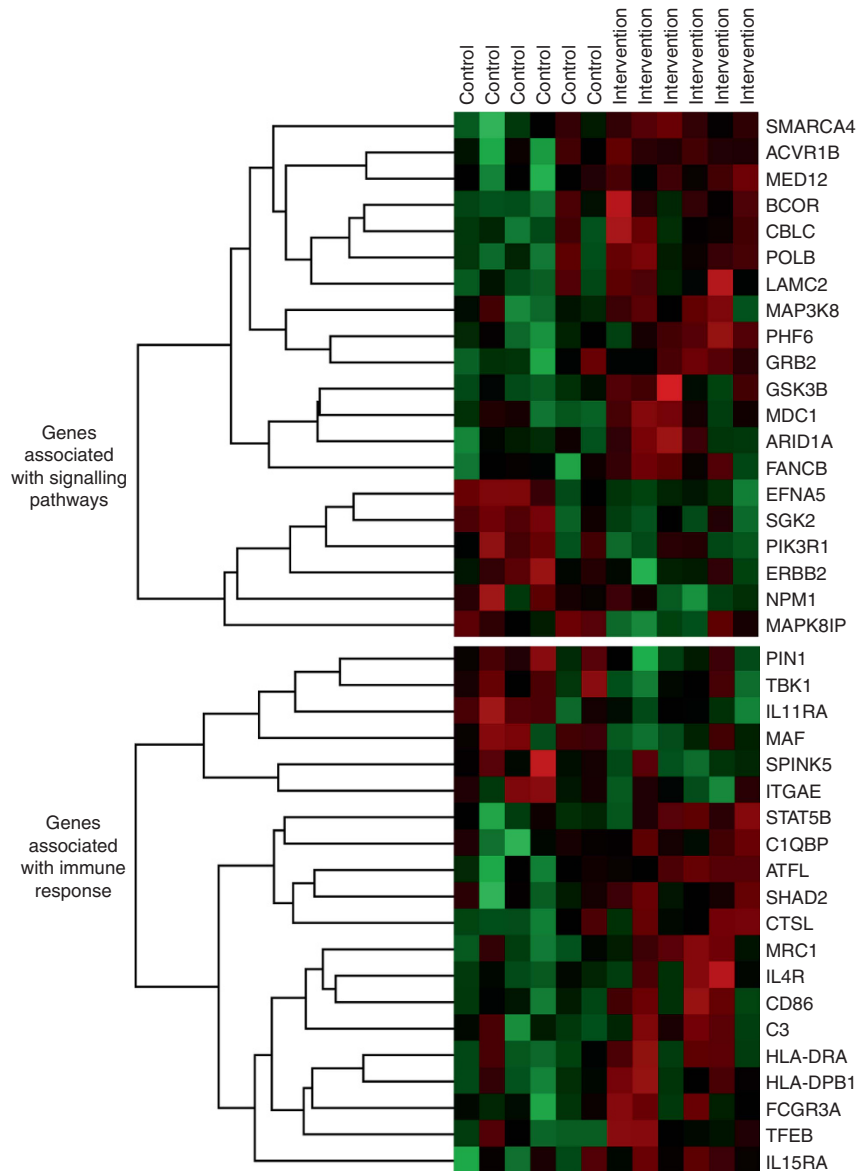
Figure 3. Heat map of the top 20 genes associated with tumour signalling and immune response within surgical tumour specimens of prostate cancer patients assigned to control (left side) *vs* weight loss (right side) before prostatectomy. Green shading indicates downregulated genes and red shading indicates upregulated genes, with shading intensity indicating departure magnitude from a known standard (black).

(Demark-Wahnefried *et al*, 2015). Albeit to meet targeted accrual, a 6-month extension was necessary; thus, future trials may require multiple sites, especially if conducted in the US where many surgeries are performed within 3 weeks of diagnosis.

CONCLUSION

Similar to most feasibility trials, this study raises more questions than it answers, particularly given the unexpected effect on tumour proliferation. Although the results must be cautiously interpreted, they do call for larger studies to replicate findings and answer questions. Specifically, when in the treatment course should weight loss be pursued and how do we optimise dose and content? What is driving increased Ki67 staining, is this associated with a detrimental increase in tumour cell proliferation or a beneficial increase in local immune activation? Some of these concerns are addressed by an American Society of Clinical Oncology report that calls for future research in obesity and cancer (Ligibel *et al*, 2015).

Until more is known, findings of this study call for caution and support observations by Caan *et al* (2012) and Lennon *et al* (2016) who describe an 'obesity paradox in cancer'. Both point to possible dangers of weight loss, with Caan *et al* (2012) recommending that cancer survivors prevent weight gain, rather than losing weight until more is known, and urging personalised weight management strategies based on medical history. Given the high prevalence of diabetes and cardiovascular disease in our sample, the potential for weight loss to mitigate these competing risks is large and most likely outweighs the threat to cancer control. Clearly, more research is needed to confirm or refute the impact of weight loss, particularly rapid weight loss, on prostate tumour biology.

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CONFLICT OF INTEREST

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