Amino Acids (2007) 33: 203–212
DOI 10.1007/s00726-007-0524-1
Printed in The Netherlands

Amino Acids

Polyamine contents in current foods: a basis for polyamine reduced diet and a study of its long term observance and tolerance in prostate carcinoma patients

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Received October 31, 2006
Accepted February 1, 2007
Published online June 20, 2007;

Summary. Polyamine contents were assessed by mass spectrometry in 233 current foods and beverages. In order to reduce gut polyamine uptake, a polyamine reduced diet (PRD) and partial intermittent intestinal tract decontamination (PIITD) with neomycin or nifuroxazide was proposed as nutritional therapy to 33 prostate carcinoma patients, 30 of whom with hormone refractory prostate cancer (HRPC). Mean PRD observance was 22 ± 19 (median: 16; range: 3–72) months. 10, 8 and 3 patients were respectively on PRD for more than 30, 36 and 64 months. No diet toxicity was observed. 8 patients had moderate intestinal intolerance due to PIITD which was interrupted. No significant differences in body weight, blood counts or serum protein levels were observed during the follow-up of patients under PRD. Performance status and pain scores were relatively stable during the trial with improved pain scores at 6 months. A PRD associated with intermittent PIITD is a safe and well observed nutritional regimen and long term observance is possible.

Keywords: Polyamines – Nutrition – Therapy – Prostate cancer – Pain – Performance status

Abbreviations: Cd, Cadaverine; DFMO, difluoro-methylornithine; FIA, flow injection analysis; HRPC, hormone refractory prostate cancer; MS, ionization-mass spectrometry; ODC, ornithine-decarboxylase; PA, polyamine; PDD, polyamine deficient diet; PIITD, partial intermittent intestinal tract decontamination; PRD, polyamine reduced diet; PSA, Prostate Specific Antigen; Pt, putrescine; RBC, red blood cell; SAM, S-adenosyl-methionine; Sd, spermidine; Sm, spermine; WBC, white blood cells

Introduction

The polyamines belong to a very wide range of biogenic amines that are involved in many physiological functions (Cohen, 1998). These ubiquitous chemical entities are believed to participate in cellular proliferation and differentiation. In mammal, cellular polyamines (i.e. putrescine, spermidine, spermine) derive from endogenous biosynthesis, as well as from the diet and from intestinal micro-organisms. Putrescine (Pt) is synthesised from ornithine by a reaction catalysed by ornithine-decarboxylase (ODC), the limiting enzyme in polyamine synthesis. Cadaverine (Cd), a polyamine chemically near enough to putrescine, is produced by micro-organisms and originates from the decarboxylation of lysine by lysine-decarboxylase. The other two mammalian polyamines derive from putrescine by successive attachment of two propylamine groups by the action of aminopropyl-transferases, namely spermidine (Sd) and spermine (Sm) synthase. The propylamine group donor is S-adenosyl-S-methyl-homocysteamine derived from S-adenosyl-methionine by the action of SAM-decarboxylase. Abnormalities in the homeostatic control of polyamines metabolism are implicated in several pathological processes, including cancer.

In vitro, difluoro-methylornithine (DFMO), an inhibitor of ODC, is an effective inhibitor of malignant cell proliferation, but in vivo the effectiveness of DFMO is reduced by tumour cell uptake of polyamines released into the circulation by normal and cancer cells and from gut flora or dietary sources. Tumour-bearing animals fed with a polyamine deficient diet (PDD) and treated by DFMO and neomycin (partial decontamination of the gastro-intestinal tract), exhibit a very significant inhibition of tumour progression and of metastasis spreading (Seiler et al., 1990), as well as an anticancer immunity stimulation (Chamaillard et al., 1997), without inducing deleterious secondary effects. Moreover, in tumour-grafted animals only fed with PDD and receiving neomycin in the drinking water (i.e. without DFMO), we have observed a 40% inhibition in tumour progression and metastasis spreading (Seiler et al., 1990). Given alone, neither neomycin nor DFMO was able to positively modify the malignant evolution.

This led us to analyse the polyamine content of common foodstuffs in an attempt to reduce the polyamine intake in cancer patients. As previously shown by Bardocz et al. (1995) some food ingredients contain large quantities of polyamines. A preliminary clinical trial performed in 14 metastatic hormone-refractory prostate cancer (HRPC) patients has revealed the feasibility of this nutrition therapy based upon a 6 months polyamine reduced diet combined with a neomycin treatment (Cipolla et al., 2003). Reducing polyamine dietary intake and partial intestinal decontamination is a well-observed and tolerated regimen, and seemed to be beneficial for patient quality of life and pain control: performance status was improved during the regimen and deteriorated 3 months after stopping it. Pain score was improved during the diet and increased 3 months after stopping. One patient had a >50% reduction in Prostate Specific Antigen (PSA), three patients had PSA stabilization for 6 months. No significant modification of other studied biological parameters was noted.

In the present work we have extended the study of polyamine contents to 233 current foods and beverages, establishing tables which have been used as non invasive nutrition therapy for the treatment of 33 prostate cancer patients. We also present the results of long term PRD concerning its observance, safety, effect on quality of life (performance status and pain control) and on main biological parameters.

Materials and methods

Polyamine extraction

Ten samples were taken from each nutriment or beverage and were homogenized in 10 vol. of ice-cold $HClO_4$ (10% v/v) in motor driven glass homogenizers and maintained for 2 h at 4 °C. The homogenates were then centrifuged at 5000 *g* for 10 min at 4 °C. Five aliquots of 1 ml from each perchloric supernatant were frozen at −80 °C until analysis. Red blood cell (RBC) polyamine extractions were performed from venous blood samples (5 ml) collected in evacuated blood collection tubes containing a 0.129 M sodium citrate solution, as previously described (Cipolla et al., 1990).

Polyamine determination

Polyamine (Pt, Cd, Sd, Sm) food and beverage contents were assessed by atmospheric pressure chemical ionization-mass spectrometry (MS) after polyamine dansylation, without chromatographic separation, as we recently described (Gaboriau et al., 2003). To each 1 ml of perchloric extract was added 500 µl of a saturated aqueous solution of Na_2CO_3 then 1 ml of a Dansyl chloride solution (5 mg/ml acetone). After shaking, the tubes were left open under a dark fume-hood to allow selective evaporation of the acetone. The dansylated derivatives were extracted twice with 1 ml each of benzene and evaporated to dryness in a rotary evaporator. The residues were dissolved in acetonitrile and subjected to MS. Positive-ions mass spectra for each dansylated polyamine were generated after optimization by flow injection analysis (FIA). FIA coupled with MS detection by selected ion monitoring greatly increased the sensitivity of the polyamine detection. The method is linear over a wide range of polyamine concentrations and allows detection of quantities as low as 5 fmol. The FIA/MS method is about 50-fold more sensitive than the conventional HPLC/fluorimetry procedure. The FIA/MS method notably reduces the time of analysis per sample to 1.5 min and turns out to be rapid, efficient, reproducible, and sufficiently simple to allow its routine application. RBC polyamine determinations were performed by FIA/MS, as previously described. In this study, the polyamine levels are shown in 13 tables and expressed in nmoles per g (food) or per ml (beverage) of putrescine, cadaverine, spermidine, spermine and sum of the four polyamines; each polyamine level is expressed as mean ± SD from at least 50 FIA/MS analysis for each food of beverage (10 samples; 5 perchloric supernatant per sample).

Patients

Of 45 consecutive patients with metastatic HRPC, 30 accepted, after informed consent and extensive explanation of the trial rationale, a polyamine (PA) reduced diet (PRD) in adjunction to their conventional treatment. The 15 other patients were excluded from the trial, either because they refused the trial, either because they did not understand the rationale, either because regular observance was not possible.

Partial intermittent intestinal tract decontamination (PIITD) was proposed one week out of two, with oral neomycin (750 mg, daily) for the first fourteen patients and then with nifuroxazide (600 mg, daily) for the other patients as neomycin was not available anymore, withdrawn by the industry. Mean time from initiation of androgen ablation to HRPC is 33 ± 26 (median: 33) months. All 30 patients with HRPC had prior PSA relapse following secondary hormonal manipulations (anti-androgen addition or withdrawal and then diethylstilbestrol), thus defining HRPC. Mean PSA at PRD entry is 267 ± 800, median 30 (range 1–4300) ng/ml. Prior treatment: 1 patient had prior systemic radio-isotope therapy, 4 patients had conventional chemotherapy with docetaxel or mitoxantrone, 8 patients had estramustine phosphate and 5 patients low dose oral cyclophosphamide (100 mg per day) + prednisone 10 mg a day. Median time from the beginning of HRPC to beginning of the PRD is 11 ± 9 (range 1–32), (median: 9) months.

Three informed patients with non metastatic disease had chosen a PRD as an alternative to androgen deprivation after local relapse to radical treatment (radical prostatectomy + adjuvant radiotherapy) and were included in the study for long term PRD evaluation.

Thus, 33 volunteers (mean age: 68 ± 10 years, range 46–82) were enrolled in this study.

A list of these foods was given to every patient who could thus prepare his meals freely. Patients were counselled to follow the diet 5 days a week but could eat what they wanted any other two days. Three groups of foods were established according to their PA contents. Group I (containing <100 nmol/g/ml PA) could be eaten ad libitum, Group II (101–200 nmol/g/ml PA) 3 or 4 times a week only, and Group III (>201 nmol/g/ml PA) foods or beverages were forbidden. Food/beverage verage quantities were equally free of choice. Toxicity and observance was noted. Regular assessment of body weight, performance and pain status was performed according to the WHO (Grade 0: able to carry out all normal activity without restriction; Grade 1: restricted in physically strenuous activity but ambulatory and able to carry out light work; Grade 2: ambulatory and capable of all self-care but unable to carry out any work;

up and about more than 50% of waking hours; Grade 3: capable of only limited self-care; confined to bed or chair more than 50% of waking hours; Grade 4 completely disabled; cannot carry on any self-care, totally confined to bed or chair) and EORTC scales (Grade 0: no antalgics; grade 1: occasionnal non morphinic antalgics; Grade 2: regular non morphinic antalgics; Grade 3: occasionnal morphinic antalgics; grade 4: regular morphinic antalgics), respectively. PSA, haemoglobin, white blood cells (WBC) and platelets blood counts, serum proteins and RBC Sd and Sm (expressed in nmol/8×10^9 erythrocytes) were also regularly evaluated. Normal RBC Sp and Sm values are respectively 8 ± 3 and 6 ± 2.5 nmol/ 8×10^9 erythrocytes.

In our initial experience, the diet was proposed for 6 months only, corresponding to the first 4 patients in this study, then afterwards, PRD was proposed as long as the patient tolerated or wished to continue it or until death. During the diet and because of increasing PSA or localized pain, 14 patients had estramustine phosphate, 4 patients palliative radiotherapy, 11 patients low dose oral cyclophosphamide (100 mg), 4 patients chemotherapy (2 mitoxantrone and 2 docetaxel).

Patients were systematically seen and examined, every three or six months, in consultation by one of the authors (B.G.C), for routine check-up. Diet observance was assessed by asking the patient and/or his wife if compliance was regular. As no efficient compliance measurement tool is available, only confidence between the patient and the urologist served as guideline.

Results are expressed as mean values ± standard deviation.

Statistics were performed with the non parametrical Wilcoxon test for comparison between paired values. The trial was approved by the local ethics committee.

Results

Tables 1–13 respectively correspond to the polyamine content of meat ($n = 14$), delicatessen ($n = 13$), fish and seafoods ($n = 16$), vegetables ($n = 33$), fruits ($n = 33$), dairy products ($n = 23$), eggs ($n = 3$), graminaceae ($n = 4$), breads ($n = 4$), desserts ($n = 17$), condiments and seasonings ($n = 15$), canned foods ($n = 19$), beverages ($n = 29$).

Table 1. Polyamine contents in meats ($n = 14$)

	Putrescine (1)	Cadaverine (2)	Spermidine (3)	Spermine (4)	Total (1 + 2 + 3 + 4)
Diced bacon	3	12.1 ± 0.1	7.0 ± 0.1	26.3 ± 0.1	48.3
Toulouse sausage	3	1	16.3 ± 0.2	46.2 ± 0.3	66.5
Spicy sausage (merguez)	8.1 ± 0.1	1	12.2 ± 0.1	48.3 ± 0.4	69.6
Chipolata	3	1	24.2 ± 0.2	50.3 ± 0.5	78.5
Frankfurter sausage	11.1 ± 0.1	14.2 ± 0.2	27.0 ± 0.2	31.1 ± 0.2	83.4
Pork (roast)	4	3	9.3 ± 0.1	72.3 ± 0.2	88.6
Turkey (wing)	14.1 ± 0.1	2.0 ± 0.1	10.1 ± 0.1	68.3 ± 0.4	94.5
Ox tongue	12.1 ± 0.1	1	45.2 ± 0.3	43.2 ± 0.2	101.5
Chicken (leg)	12.2 ± 0.1	bdl	92.1 ± 0.3	8.1 ± 0.1	112.1
Rabbit (leg)	5.1 ± 0.1	1	52.2 ± 0.3	76.3 ± 0.5	134.6
Veal	41.5 ± 2.1	1	27.4 ± 0.7	100.6 ± 0.7	169.4
Lamb	8.2 ± 0.1	bdl	39.7 ± 0.7	131.3 ± 2.1	178.3
Chicken (wing)	8.0 ± 0.2	bdl	64.3 ± 0.9	114.7 ± 2.2	186.9
Beef	30.5 ± 0.1	bdl	17.5 ± 0.4	140.8 ± 2.1	187.7

Values expressed in nmol/g ± SD. *Bdl* Below detection limits

Table 2. Polyamine contents in delicatessen ($n = 13$)

	Putrescine (1)	Cadaverine (2)	Spermidine (3)	Spermine (4)	Total (1 + 2 + 3 + 4)
Bacon	2	12.1 ± 0.1	6	26.2 ± 0.2	46.3
Chorizo	15.0 ± 0.1	4	15.0 ± 0.1	40.4 ± 0.5	74.4
Potted minced pork	4	3	17.9 ± 0.1	53.9 ± 0.3	78.8
Saveloy	5	27.0 ± 0.2	14.7 ± 0.1	38.8 ± 0.2	85.5
Salami	6	22.8 ± 0.3	21.5 ± 0.2	44.9 ± 0.2	95.2
Ham	5	26.1 ± 0.2	8.1 ± 0.1	63.4 ± 0.5	102.6
Smoked ham	10.0 ± 0.1	11.3 ± 0.1	22.4 ± 0.3	84.5 ± 0.6	128.2
Garlic sausage	7	66.8 ± 0.2	31.5 ± 0.3	45.5 ± 0.5	150.8
Liver mousse	5	49.2 ± 0.2	56.9 ± 0.3	115.4 ± 0.9	226.5
Chitterlings	7	4	143.2 ± 0.8	93.4 ± 0.5	247.6
Duck-liver pâté	21.1 ± 0.2	85.3 ± 0.5	106.2 ± 0.7	132.1 ± 0.5	344.7
Pork-liver pâté	11.4 ± 0.1	186.7 ± 1.2	59.9 ± 0.4	110.4 ± 0.5	368.4
Rosette (gamy pork sausage)	1025.6 ± 10.1	473.2 ± 0.9	13.0 ± 0.1	62.9 ± 0.5	1574.7

Values expressed in nmol/g ± SD. *Bdl* Below detection limits

Table 3. Polyamine contents in fish and seafoods ($n = 16$)

	Putrescine (1)	Cadaverine (2)	Spermidine (3)	Spermine (4)	Total (1 + 2 + 3 + 4)
Langoustine, scampi	2.3 ± 0.1	2.6 ± 0.1	0.1	bdl	5.0
Shrimp	2.7 ± 0.1	5.3 ± 0.1	2.4 ± 0.1	bdl	10.4
Crayfish	1	7.0 ± 0.1	4.0 ± 0.1	bdl	12.0
Hake (fresh)	10.0 ± 0.2	bdl	2.0 ± 0.1	1	13.0
Cod	15.0 ± 0.1	1	1	bdl	17.0
Hake (frozen)	24.0 ± 0.2	1	1	1	27.0
Whiting	15.1 ± 0.1	47.1 ± 0.5	6.1 ± 0.1	8.5 ± 0.1	76.7
Scottish smoked salmon	14.3 ± 0.1	49.3 ± 0.6	13.2 ± 0.1	12.0 ± 0.1	88.8
Red mullet	62.0 ± 1.5	50.0 ± 1.5	10.0 ± 0.1	14.0 ± 0.1	136.0
Salmon (fresh)	44.0 ± 1.2	1	60.0 ± 2.0	45 ± 0.7	150.0
Calamari, squid	152.0 ± 7.8	47.5 ± 0.6	2.0 ± 0.1	15.0 ± 0.5	216.5
Oysters	11.3 ± 0.1	67.1 ± 3.1	167.2 ± 8.9	105.8 ± 8.1	351.4
Muscles	11.7 ± 0.1	58.3 ± 2.1	259.5 ± 5.6	129.8 ± 7.1	459.3
Crab claw	11.9 ± 0.1	562.3 ± 7.2	1.4 ± 0.1	bdl	575.5
Scallops (white)	285.7 ± 6.1	2111.6 ± 12	6.8 ± 0.1	14.0 ± 0.1	2418.3
Scallops (coral)	487.3 ± 7.1	3849.4 ± 15	14.5 ± 0.2	49.9 ± 0.5	4401.9

Values expressed in nmol/g ± SD. *Bdl* Below detection limits

Table 4. Polyamine contents in vegetables ($n = 33$)

	Putrescine (1)	Cadaverine (2)	Spermidine (3)	Spermine (4)	Total (1 + 2 + 3 + 4)
Salsify	1	bdl	10.1 ± 0.1	1	12.1
Onion	5.7 ± 0.2	3.3 ± 0.1	35.4 ± 0.5	bdl	44.3
Celery	25.0 ± 0.2	0.1	22.0 ± 0.2	0.7	47.8
Normal ripe carrot	7.7 ± 0.1	0.8 ± 0.1	53.6 ± 0.3	2.7 ± 0.1	64.8
Green cabbage	6.0 ± 0.1	1	55.7 ± 0.2	6.4 ± 0.1	69.1
Beet, beetrout	51.7 ± 0.5	0.2	29.1 ± 0.1	bdl	81.0
Skinned potato	31.4 ± 0.2	5.1 ± 0.1	46.1 ± 0.3	4.3 ± 0.1	86.8
Gratted carrot	28.3 ± 0.2	6.0 ± 0.1	52.9 ± 0.4	0.6	87.8
Sorrel	5	bdl	92.7 ± 0.3	bdl	97.7
French (string) beans	65.2 ± 0.3	bdl	27.2 ± 0.1	6.0 ± 0.1	98.4
Red bean	6.0 ± 0.1	bdl	78.3 ± 0.5	24.2 ± 0.2	108.5
Radish	10.5 ± 0.1	12.7 ± 0.1	87.6 ± 0.3	1.7 ± 0.1	112.5
Chicory	65.3 ± 0.4	1.1	52.6 ± 0.2	1.1	120.1
Leek (white)	24.6 ± 0.2	4.5 ± 0.1	82.2 ± 0.3	9.1 ± 0.1	120.5
Escarolle (endive)	62.0 ± 0.3	1.9 ± 0.1	69.6 ± 0.5	3.0 ± 0.1	136.4
Red cabbage	75.8 ± 0.5	1.9 ± 0.1	60.1 ± 0.4	4.5 ± 0.1	142.2
Onion shallot	31.7 ± 0.3	4.4 ± 0.1	101.8 ± 0.5	8.9 ± 0.1	146.8
Potato with skin	64.8 ± 0.5	7.7 ± 0.2	71.0 ± 0.5	4.4 ± 0.1	148.0
Potato starch	64.6 ± 0.2	3.0 ± 0.1	79.5 ± 0.3	13.1 ± 0.1	160.2
Leek (green)	30.2 ± 0.2	4.1 ± 0.1	127.2 ± 0.8	12.3 ± 0.2	173.8
Peeled Spring potato	94.7 ± 0.5	8.7 ± 0.1	81.5 ± 0.5	5.5 ± 0.1	190.4
Spring potato with peel	87.7 ± 0.3	9.8 ± 0.1	89.9 ± 0.4	8.2 ± 0.1	195.7
Brussels sprout	59.1 ± 0.2	bdl	127.6 ± 1.1	9.1 ± 0.1	195.8
Lettuce	89.4 ± 0.4	5.2 ± 0.1	95.6 ± 0.5	5.9 ± 0.1	196.1
Garlic	13.1 ± 0.1	5.8 ± 0.1	155.3 ± 0.9	33.8 ± 0.5	208.0
Chervil	107.5 ± 1.6	bdl	123.7 ± 1.6	8	239.2
Tarragon	125.6 ± 1.7	1	135.7 ± 1.6	14.1 ± 0.6	276.4
Cabbage	74.9 ± 0.6	4.7 ± 0.1	195.3 ± 0.9	18.1 ± 0.2	293.1
Broccoli	64.2 ± 0.3	6.5 ± 0.1	214.0 ± 0.9	22.8 ± 0.7	307.5
Parsley	114.3 ± 2.6	1	195.2 ± 2.9	19.9 ± 0.6	330.4
Mushroom	4.0 ± 0.1	1.5 ± 0.1	450.0 ± 2.2	3.0 ± 0.1	458.5
Button mushroom	133.5 ± 0.6	5.1 ± 0.1	398.5 ± 0.7	3.1 ± 0.1	540.2
Garden peas	196.5 ± 0.7	2	348.4 ± 0.8	19.1 ± 0.2	566.0

Values expressed in nmol/g ± SD. *Bdl* Below detection limits

Table 5. Polyamine contents in fruits ($n = 33$)

	Putrescine (1)	Cadaverine (2)	Spermidine (3)	Spermine (4)	Total (1 + 2 + 3 + 4)
Raisins	2	1	3	1	6.0
Apple (Junagored)	5.1 ± 0.1	1	7.1 ± 0.1	bdl	13.2
Prune	6.1 ± 0.1	1	10.1 ± 0.1	2	19.2
Pear (William)	0.4 ± 0.1	0.7	18.4 ± 0.1	0.2	19.8
Apple (Golden)	7.4 ± 0.1	0.7	15.0 ± 0.1	bdl	24.1
Avocado	3.1 ± 0.1	1.9 ± 0.1	12.7 ± 0.1	6.6 ± 0.1	24.3
Pear (Conference)	4.3 ± 0.1	0.7	20.3 ± 0.1	bdl	25.3
Peach	7.4 ± 0.7	1	18.4 ± 0.2	bdl	26.8
Yellow-fleshed peach	8.6 ± 0.1	1	19.4 ± 0.2	bdl	29.3
Date (dry)	32.2 ± 0.2	bdl	10.1 ± 0.1	1	43.3
Pineapple	7.6 ± 0.1	0.8	27.0 ± 0.2	10.9 ± 0.1	46.3
Tomato (bunch)	14.4 ± 0.1	8.6 ± 0.1	24.7 ± 1.1	bdl	47.8
Yellow pepper	14.3 ± 0.2	5.5 ± 0.1	27.5 ± 0.2	3.7 ± 0.1	51.0
Apple (Grany)	30.3 ± 0.7	0.9 ± 0.1	22.1 ± 0.2	bdl	53.3
Red grapes	34.2 ± 0.6	6.4 ± 0.1	15.2 ± 0.2	0.1	55.8
Green grapes	18.3 ± 0.3	5.0 ± 0.1	29.8 ± 0.2	3.1 ± 0.1	56.2
Kiwi	13.3 ± 0.1	1.3 ± 0.1	37.3 ± 0.3	7.4 ± 0.1	59.0
Green pepper	21.6 ± 0.2	5.3 ± 0.1	30.4 ± 0.2	4.1 ± 0.1	61.4
Lemon	53.8 ± 0.3	0.8 ± 0.1	18.4 ± 0.1	0.9 ± 0.1	73.8
Strawberry	18.5 ± 0.1	4.3 ± 0.1	40.3 ± 0.3	13.7 ± 0.1	76.8
Red pepper	26.9 ± 0.2	12.9 ± 0.1	32.4 ± 0.2	8.4 ± 0.2	80.6
Small tomato (cherry)	26.3 ± 0.5	15.4 ± 0.3	52.1 ± 0.9	3.2 ± 0.1	96.9
Melon	22.7 ± 0.2	1	77.7 ± 0.7	4.9 ± 0.1	106.3
Cucumber	98.9 ± 0.5	1.8 ± 0.1	65.0 ± 0.7	1.3 ± 0.1	167.0
Hazelnut (dried)	47.7 ± 0.3	2	144.5 ± 2.9	32.3 ± 0.9	226.5
Almond (grilled nut)	35.6 ± 0.2	2	207.4 ± 3.1	54.2 ± 0.8	270.2
Pistachio (grilled nut)	87.4 ± 0.5	5	208.6 ± 2.5	54.3 ± 0.5	355.3
Banana	317.3 ± 4.1	7.5 ± 0.1	44.9 ± 0.5	1	370.9
Aubergine, eggplant	360.0 ± 7.2	3.0 ± 0.1	30.0 ± 0.2	3.0 ± 0.1	396.0
Normal ripe tomato	381.1 ± 6.1	10.1 ± 0.1	19.4 ± 0.4	bdl	410.4
Peanut (grilled nut)	61.4 ± 1.5	4	388.7 ± 2.5	34.6 ± 0.5	488.7
Marrow, courgette	393.5 ± 5.2	18.4 ± 0.2	107.0 ± 5.2	7.5 ± 0.2	526.4
Orange	1047.7 ± 9.1	24.5 ± 0.9	17.8 ± 0.2	bdl	1090.0

Values expressed in nmol/g ± SD. *Bdl* Below detection limits

Noticeable differences are observed in the food and beverage polyamine contents. Some common foodstuffs, for example orange and orange juice (Table 5 and 13 respectively), contain very high polyamine levels.

Observance and toxicity: Mean PRD observance was 22 ± 19; median: 16 (range 3–72) months. No patient stopped the diet due to intolerance or weariness.

Long term PRD: 10 patients continued a PRD for at least 30 months, 8 for at least 36 months, 4 for 42 months and 3 for at least 64 months. These three last patients are still alive and working normally. No adverse effects due to the diet was observed.

Eight patients (24%) stopped neomycin or nifuroxazide, 5 months (median) after initiation, because of intestinal intolerance (mild diarrhae or flatulence). They continued with PRD alone.

Body weight, performance status, pain status, serum haemoglobin, white blood cell count, serum protein, alkaline phosphatases, P.S.A., erythrocyte Sd and Sm levels results are reported in Table 14.

No significant difference in all studied variables and platelet count was observed.

Discussion

To our knowledge, we present the first study of polyamine concentrations measured by mass spectrometry, 50 fold more sensitive than standard High Pressure Liquid Chromatography. As shown in the foodstuffs tables, polyamine concentrations vary considerably in food. By limiting dietary intake to foodstuffs with preferably low (<100 nmoles per g) or occasionally intermediate (101–200 nmoles per g) polyamine concentrations, we estimate that exogenous dietary polyamine intake can be reduced 20-fold. In this study, the number of foods and beverages assessed for polyamine content has been increased

Table 6. Polyamine contents in dairy products ($n = 23$)

	Putrescine (1)	Cadaverine (2)	Spermidine (3)	Spermine (4)	Total (1 + 2 + 3 + 4)
Butter	bdl	bdl	bdl	bdl	bdl
Salted butter	bdl	bdl	1	bdl	1
Cream	1	bdl	bdl	bdl	1
Milk (semi-skinned, low fat)	0.3	bdl	0.4	0.4	1.1
Yoghurt natural	bdl	1	1	1	3
Soft cheese	0.4	1.8 ± 0.1	1.4 ± 0.1	0.1	3.7
Swiss Emmental	1.5	1.7 ± 0.1	3.7 ± 0.1	1.6 ± 0.1	8.5
Strawberry flavoured yoghurt	3	1	5	bdl	9
French Emmental	5	1.8 ± 0.1	4.3 ± 0.1	2.5 ± 0.1	13.6
Mixed herbs soft cheese	3	2.7 ± 0.1	6.6 ± 0.1	2	14.3
Powdered milk	1.5	bdl	7	7	15.5
Goat's cheese without rind	7.2 ± 01	4.5 ± 0.2	4.2 ± 0.1	1.6 ± 0.1	17.4
Brie pasteurised without rind	1.7	8.3 ± 0.4	5.1 ± 0.1	4.7 ± 0.2	19.7
Grated cheese	8.0 ± 01	7.0 ± 0.1	3.1 ± 0.1	8.1 ± 0.1	26.2
Camembert	9.9 ± 01	19.1 ± 0.7	1.1	1.1	31.1
Brie pasteurised with rind	14.7 ± 0.7	1.5 ± 0.1	58.0 ± 0.9	2.3 ± 0.2	76.5
Goat's cheese with rind	17.6 ± 0.5	4.2 ± 0.2	111.8 ± 3.1	8.8 ± 0.5	142.3
Sweet Cantal without rind	19.5 ± 0.5	166.3 ± 9.7	1.1	0.9	187.7
Roquefort	54.8 ± 2.1	116.3 ± 6.1	141.9 ± 4.1	0.8	313.9
Sweet Cantal with rind	46.2 ± 1.2	534.2 ± 9.7	26.7 ± 0.8	11.4 ± 0.1	618.5
Comté	124.6 ± 3.1	2165.6 ± 10	2.0 ± 0.1	1.2 ± 0.1	2293.4
Saint Nectaire without rind	736.3 ± 7.1	3929.9 ± 12	22.0 ± 0.5	5.6 ± 0.1	4693.8
Saint Nectaire with rind	819.9 ± 7.8	4470.8 ± 12	0.8	3.4 ± 0.1	5294.8

Values expressed in nmol/g or nmol/ml ± SD. *Bdl* Below detection limits

Table 7. Polyamine contents in eggs ($n = 3$)

	Putrescine (1)	Cadaverine (2)	Spermidine (3)	Spermine (4)	Total (1 + 2 + 3 + 4)
Egg white (freshly-laid)	1	bdl	bdl	bdl	1.0
Freshly-laid egg	20.5 ± 0.1	bdl	bdl	bdl	20.5
Egg yolk (freshly-laid)	43.7 ± 0.1	bdl	bdl	bdl	43.7

Values expressed in nmol/ml ± SD. *Bdl* Below detection limits

Table 8. Polyamine contents in graminaceae ($n = 4$)

	Putrescine (1)	Cadaverine (2)	Spermidine (3)	Spermine (4)	Total (1 + 2 + 3 + 4)
Rice (white)	2.0 ± 0.1	bdl	bdl	bdl	2.0
Semolina	25.1 ± 0.1	1	5.0 ± 0.1	23.1 ± 0.2	54.2
Fresh pasta	13.0 ± 0.2	3	35.1 ± 0.1	6.0 ± 0.1	57.1
Soft wheat	60.0 ± 0.3	20.1 ± 0.1	105.3 ± 0.6	45.4 ± 0.2	230.8

Values expressed in nmol/g ± SD. *Bdl* Below detection limits

Table 9. Polyamine contents in breads ($n = 4$)

	Putrescine (1)	Cadaverine (2)	Spermidine (3)	Spermine (4)	Total (1 + 2 + 3 + 4)
White bread	13.0 ± 0.1	15.3 ± 0.1	54.2 ± 0.4	13.2 ± 0.1	95.7
Oat bread	22.1 ± 0.1	13.2 ± 0.1	65.4 ± 0.4	12.6 ± 0.2	113.3
Rye bread	42.5 ± 0.2	19.3 ± 0.2	86.1 ± 0.4	20.7 ± 0.2	168.6
Whole bread	40.6 ± 0.3	19.1 ± 0.2	109.3 ± 0.6	22.4 ± 0.2	191.4

Values expressed in nmol/g ± SD

Table 10. Polyamine contents in desserts ($n = 17$)

	Putrescine (1)	Cadaverine (2)	Spermidine (3)	Spermine (4)	Total (1 + 2 + 3 + 4)
Caster sugar	3	bdl	bdl	bdl	3.0
Very thin pancake (crêpe)	bdl	1	5	1	7.0
Chocolate eclair	2	2	3	bdl	7.0
Honey	8	1	1	bdl	10.0
Cookie (sweet biscuit)	2	1	6	1	10.0
Pound cake	3	1	6	1	11.0
Milk chocolate	3	3	6	1	13.0
Lemon pie	3	1	9	1	14.0
Brittany cake	5	1	10.2 ± 0.1	1	17.1
Apricot marmalade	10.4 ± 0.1	1	11.3 ± 0.1	bdl	22.4
Strawberry pie	5	3	13.2 ± 0.1	3	24.2
Raspberry marmalade	14.6 ± 0.1	1	12.0 ± 0.1	1	28.6
Strawberry marmalade	16.2 ± 0.1	bdl	13.1 ± 0.1	2	31.3
Brittany crêpes (wheat)	3	5	25.4 ± 0.2	4	37.4
Black chocolate 80% cocoa	9	7	21.5 ± 0.2	3	40.5
Fruit salad	27.6 ± 0.2	1	15.3 ± 0.1	1	44.9
Prune marmalade	52.5 ± 0.2	1	13.7 ± 0.1	2	69.2

Values expressed in nmol/g ± SD. *Bdl* Beyond detection limits

Table 11. Polyamine contents in condiments, seasonings ($n = 15$)

	Putrescine (1)	Cadaverine (2)	Spermidine (3)	Spermine (4)	Total (1 + 2 + 3 + 4)
Salt	bdl	bdl	bdl	bdl	bdl
White pepper	bdl	bdl	bdl	bdl	bdl
Salad oil	bdl	bdl	bdl	bdl	bdl
Béarnaise sauce	bdl	3	3	1	7.0
Wine vinegar	8	bdl	bdl	bdl	8.0
Mayonnaise	1	3	4	1	9.0
Mustard sauce	5	1	10.4 ± 0.1	1	17.4
Garlic mayonnaise	3	3	12.1 ± 0.1	1	19.1
Tartar sauce	12.1 ± 0.1	1	11.3 ± 0.1	1	25.4
Bourguignon sauce	53.6 ± 0.3	3	12.2 ± 0.1	2	70.8
Vinegar ancient style	79.5 ± 0.3	5	bdl	bdl	84.5
Ketchup	107.6 ± 0.5	4	22.3 ± 0.1	2	137.9
Mustard	20.4 ± 0.2	1	235.9 ± 0.8	9	266.3
Tinned gherkin	96.8 ± 0.2	1	200.0 ± 1.9	18.7 ± 0.2	316.5
Tomato concentrate	550.8 ± 3.1	18.9 ± 0.1	85.7 ± 0.5	bdl	655.4

Values expressed in nmol/g or nmol/ml ± SD. *Bdl* Below detection limits

($n = 223$) compared to our previous report (Cipolla et al., 2003). No food class is excluded. The variety of "authorized" meat, poultry, fish, dairy products, vegetables, fruits, condiments and oils contributes to a balanced diet and offers patients a wide variety of choice for their meal preparation. The variety of products, the possibility to freely fix menus and eat "normally" two days a week, probably explains the high patient observance. No drop outs due to side-effects or patients growing gastronomically tiresome were recorded. Furthermore, when questioned on the diet, patients did not consider it too bothersome. When patients are on long term PRD (11 have been on the diet for more than 2 years), they do not consider interrupting it.

No diet specific toxicity was observed. Only moderate intestinal toxicity was reported due to long term intestinal tract decontamination by neomycin or nifuroxazide. Body weight was stable and no studied biological variable was significantly impaired during the diet, particularly in long term PRD patients.

Prostate cancer has a particular evolution. It is slow growing and indolent for many years in its early stages but when it evolves unfavourably and escapes hormonal control (androgen deprivation), outcome for patients

Table 12. Polyamine contents in tinned foods ($n = 19$)

	Putrescine (1)	Cadaverine (2)	Spermidine (3)	Spermine (4)	Total (1 + 2 + 3 + 4)
Apple stew	5	1	7	bdl	13.0
Potted mince from tuna	2	2	6	10.0 ± 0.1	20.0
Potted mince from salmon	1	8.3 ± 0.1	11.3 ± 0.1	10.3 ± 0.1	30.9
Vegetable soup	24.5 ± 0.1	6	35.7 ± 0.3	5	71.2
Beef tongue	12.4 ± 0.1	1	45.3 ± 0.4	43.4 ± 0.3	102.1
Paella	40.2 ± 0.4	15.3 ± 0.3	29.3 ± 0.2	20.2 ± 0.3	105.0
Chestnut	8.1 ± 0.1	3	85.3 ± 0.2	14.0 ± 0.1	110.4
Diced carrots	54.0 ± 0.2	bdl	61.0 ± 0.2	1	116.0
Mixed vegetables	39.1 ± 0.2	2	106.3 ± 0.7	14.0 ± 0.1	161.4
Peeled tomato	163.1 ± 0.3	3	34.2 ± 0.3	1	201.3
Instant mashed potato	87.4 ± 0.2	bdl	120.7 ± 0.9	2	210.1
Minced spinach	87.4 ± 0.2	1	117.5 ± 0.8	6	211.9
Tender wheat	60.3 ± 0.3	19.6 ± 0.1	104.7 ± 0.7	45.1 ± 0.3	229.7
Lentil	58.7 ± 0.2	1	163.9 ± 0.9	11.1 ± 0.1	234.7
Chickpea	29.4 ± 0.1	1	198.3 ± 0.7	6	246.4
Mixed garden pea/carrot	123.2 ± 0.2	3	179.2 ± 0.9	11.6 ± 0.2	326.0
Ratatouille	140.2 ± 0.9	1	210.0 ± 0.8	4	355.2
Garden pea	196.3 ± 2.2	2	348.4 ± 0.2	19.1 ± 0.2	565.8
Sauerkraut	1900.3 ± 8.9	130.4 ± 2.4	28.5 ± 0.5	7	2066.2

Values expressed in nmol/g ± SD. *Bdl* Below detection limits

Table 13. Polyamine contents in beverages ($n = 29$)

	Putrescine (1)	Cadaverine (2)	Spermidine (3)	Spermine (4)	Total (1 + 2 + 3 + 4)
Black coffee	bdl	bdl	bdl	bdl	bdl
Ceylon tea	bdl	bdl	bdl	bdl	bdl
Champagne cider	bdl	bdl	bdl	bdl	bdl
Cola beverage	bdl	bdl	bdl	bdl	bdl
Curdled milk	bdl	bdl	bdl	bdl	bdl
Skimmed milk	bdl	bdl	bdl	bdl	bdl
Whisky	bdl	bdl	1	bdl	1
Cognac	bdl	bdl	1	bdl	1
Indian tonic	1	bdl	bdl	bdl	1
Pastis (anisated drink)	1	4	bdl	bdl	5
Port	7.0 ± 0.1	bdl	1	bdl	8.1
White wine (Burgundy)	9.0 ± 0.1	bdl	1	bdl	10.1
Apple juice	11.1 ± 0.1	bdl	2	bdl	13.1
Grape juice	9.0 ± 0.1	bdl	5.1 ± 0.1	bdl	14.1
Apricot juice	8.0 ± 0.1	bdl	7.0 ± 0.1	bdl	15.0
Dried milk	2.0	bdl	7.0 ± 0.1	7.0 ± 0.1	16.0
Lager beer	16.0 ± 0.1	2	1	bdl	19.0
White wine (Loire)	13.1 ± 0.1	4.0 ± 0.1	3	bdl	20.1
Stout beer	21.2 ± 0.1	1	bdl	bdl	22.2
Red table wine (Bordeaux)	22.3 ± 0.1	bdl	bdl	bdl	22.3
Red wine (Côtes-du-Rhône)	26.6 ± 0.1	bdl	1	bdl	27.6
Cocktail of tropical fruits	30.1 ± 0.1	bdl	1	bdl	31.1
Liqueur wine	35.4 ± 0.1	1	bdl	bdl	36.4
Red claret (Bordeaux)	39.1 ± 0.1	1	2	bdl	42.1
Red wine (Touraine)	41.4 ± 0.2	1	5	bdl	47.4
Tomato juice	35.5 ± 0.2	1	13.2 ± 0.1	1	50.7
Red wine (Beaujolais)	48.4 ± 0.2	2	3	bdl	53.4
Grapefruit juice	99.2 ± 0.2	1	6	bdl	106.2
Orange juice	159.8 ± 0.2	1	6	bdl	166.8

Values expressed in nmol/ml ± SD. *Bdl* Below detection limits

Table 14. Body weight (kg), performance WHO status, pain EORTC status, serum hemoglobin (g/dl), white blood cell count (/ml), serum protein (g/l), Alkaline phosphatases (U.I), erythrocyte spermidine (Sd), spermine (Sm) (nmol/8.10^9 erythrocytes) and PSA (ng/ml) level results. Mean values ± SD

	M0	M3	M6	M12	M18	M24	M36	M42
n	33	33	27	20	14	11	8	4
Weight	71 ± 12	74 ± 12	76 ± 12	80 ± 10	80 ± 11	77 ± 12	78 ± 10	77 ± 11
WHO	0.5 ± 0.7	0.3 ± 0.6	0.5 ± 0.8	0.8 ± 1*	0.4 ± 0.8	0.3 ± 0.5	0.5 ± 0.8	1.5 ± 2
EORTC	0.8 ± 1.2	0.8 ± 1.2	0.4 ± 0.8*	1.3 ± 1.4*	0.6 ± 1	0.6 ± 0.8	1 ± 1.4	0.25 ± 0.5
Hb	11.8 ± 1.6	11.6 ± 2	11.8 ± 2	11.7 ± 2	12.3 ± 2	13 ± 1.5	12.5 ± 2	12.6 ± 2
WBC	6850	6560	6842	5730	6519	5667	5528	5600
Proteins	66 ± 6	65 ± 8	66 ± 8	65 ± 7	66 ± 9	70 ± 8	70 ± 3	70 ± 3
A.P	220 ± 222	328 ± 200	377 ± 600	118 ± 151	79 ± 27	93 ± 55	192 ± 319	80 ± 20
Sd	8.7 ± 5	7.4 ± 4	7.2 ± 3	7 ± 3	7 ± 2	8 ± 2,6	8.4 ± 3	9 ± 2
Sm	4.3 ± 3	4.4 ± 5	3.7 ± 2	2.4 ± 1.5	2.3 ± 1	3.1 ± 1	2.8 ± 0.7	2.8 ± 0.5
PSA	260 ± 790	500 ± 1500	374 ± 1360	443 ± 1660	69 ± 89	31 ± 60	30 ± 32	36 ± 39
median	30	51*	29*	34*	28*	14*	10	60

* $p < 0.05$ for paired values compared with M0 values

becomes grim with mean survival times varying from 12 to 18 months (Petrylak, 2002). At this stage, only palliative treatment can be proposed, focused on quality of life and pain control as modern chemotherapy regimens, although statistically improving survival time for two months, have not yet radically prolonged it.

Proposing a nutritional therapeutical regimen in cancer is a novel approach. Up to now, dietary (i.e.: vitamins and hypercaloric) complements were given to patients with a poor performance status to implement other treatments. Accepting such a dietary protocol, when other treatments have failed, is not obvious for patients, especially when life expectancy is presumably short. Conversely, when accustomed to the diet, patient observance is remarkably high.

Polyamine metabolism is a potential target in prostate cancer treatment. High polyamine levels are present in prostate tumors and in circulating red blood cells (Cipolla et al., 1990, 1993). Our initial study with 14 patients has shown that PRD was readily accepted by patients, mainly due to it's beneficial effect on performance status and pain control. When patients stopped the diet, pain increase and performance status degradation were significant (Cipolla et al., 2003). That is why in this study, latter enrolling patients were counselled to keep on following the PRD.

As previously shown, performance status is stable, throughout the diet, staying at initial WHO scores which are arguably quite low for HRPC patients. That is because, as our experience increased, PRD was proposed in the more early stages of HRPC when patients were not ≪yet≫ symptomatic but only had biological PSA relapse. The efficiency of this "pro-active" attitude is currently evaluated.

Pain control is quite remarkably favourable in a group of patients, most of which have bone metastasis in hormonal escape. Nonetheless, a majority of these patients experience disease progression during the study and eventually die from cancer ($n = 20$; 60%) but this progression does not seem to be paralleled with performance or pain status degradation, except in the late stages of the disease.

Maintaining good performance status and pain control certainly contributes to better quality of life and may also explain excellent PRD observance. Finally, PRD does not interfere with other treatments and patients who subsequently underwent chemotherapy, did not present enhanced chemotherapy side-effects.

Conclusions

A polyamine reduced diet with intermittent partial intestinal tract decontamination is a safe and a well observed nutritional regimen for selected prostate cancer patients. Long term observance is possible with no specific side effects. Pain is well controlled and performance status maintained. Reducing polyamine dietary and intestinal pools can safely be proposed to prostate cancer patients and certainly warrants further investigation.

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