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Regular ingestion of opuntia robusta lowers oxidation injury

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Summary The influence of opuntia robusta (prickly pear), a traditionally used dietary nutrient against diabetes mellitus among the American Indian population, was examined in 15 young patients suffering from familial heterozygous isolated hypercholesterolemia. Oxidation injury was determined via 8-epi-$PGF_{2\alpha}$ in plasma, serum and urine. Daily consumption of 250 g broiled edible pulp of prickly pear had no influence on body weight and body fat composition. Total cholesterol was lowered ($P < 0.01$) as was LDL-cholesterol ($P < 0.04$). No significant changes were observed either in triglycerides or in HDL. Prickly pear induced a significant decrease in plasma ($27.9 \pm 3.3 \rightarrow 25.6 \pm 3.2$; $P < 0.03$), serum ($302.0 \pm 11.4 \rightarrow 283.2 \pm 14.5$; $P < 0.0003$) and urinary ($355.9 \pm 18.4 \rightarrow 323.9 \pm 16$; $P < 0.00002$) 8-epi-$PGF_{2\alpha}$ values. The findings on a decrease of 8-epi-$PGF_{2\alpha}$ were more pronounced in females than in males, the highest significance being found in urine, while, in contrast, the effects on total- and LDL-cholesterol were more pronounced in males. A prerunning 4 weeks period of dietary counseling had no significant effect on either of the parameters examined. These findings indicate that the regular ingestion of opuntia robusta is able to significantly reduce in-vivo oxidation injury in a group of patients suffering from familial hypercholesterolemia. This traditional food of the American Indians thus may have a significant cardiovascular benefit. © 2001 Harcourt Publishers Ltd

INTRODUCTION

The value of dietary fiber in decreasing total and LDL-cholesterol and in parallel the associated cardiovascular risk is well accepted. Pima Indians in the South of the United States are well known to have the highest prevalence of metabolic syndrome and diabetes mellitus. For generations they have been using the edible pulp of the prickly pear produced by different cacti (mainly opuntia robusta or opuntia streptacantha) as a hypoglycemic compound.[1] In an earlier communication[2] we found that prickly pear ingestion decreases cholesterol, an effect which may well be explained by the pectin content of the cactus.[3] This study was designed to examine whether regular ingestion of prickly pear exerts an effect on oxidation injury in young patients suffering from familial isolated hypercholesterolemia (FH) and on oxidation injury as determined via 8-epi-$PGF_{2\alpha}$[4] in various compartments.

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PATIENTS AND METHODS

Fifteen patients suffering from isolated severe FH proven at the receptor level detected 1–6 years ago were examined (for patients, characteristics see Table 1) before entering. Although aware of the FH for various periods of time, the patients had not received drug treatment before. Basic investigation consisting of the examination of lipids (cholesterol, triglycerides) and lipoproteins (LDL, HDL) as well as the isoprostanes (8-epi-$PGF_{2\alpha}$) in plasma, serum and urine was performed. Blood was drawn after an overnight fasting period of at least 12 hours. Apart from hypercholesterolemia none of the patients had any risk factor for the development of atherosclerosis. None of the patients was a smoker. They had not taken any

Table 1 Individual patients' characteristics at study entry

No	Initials	Age (a)	Sex (m/f)	Height (cm)	Weight (kg)	Body fat (%)	Disease duration (a)
1	GD	39	m	184	82.0	21.4	4
2	KR	26	m	179	80.5	22.7	2
3	WL	34	f	166	60.0	25.3	6
4	KK	31	f	171	66.0	27.0	1
5	KR	40	m	177	75.5	24.2	3
6	IE	24	f	165	61.5	26.0	5
7	SS	29	f	163	62.5	28.2	4
8	IK	25	f	168	62.5	25.1	2
9	LP	27	m	180	81.0	22.9	5
10	PA	40	m	173	75.5	23.6	3
11	AL	36	f	167	60.0	24.7	1
12	MM	45	f	174	68.5	25.1	4
13	VT	31	m	188	86.5	20.4	2
14	MW	29	f	172	61.5	22.5	3
15	XO	42	m	180	77.0	19.3	3
$\bar{x}$	–			173.8	70.7	23.9	3.2
range		24–25	7 m/8 f	163–188	60.0–86.5	19.2–28.2	1–6

(a): years; m: male; f: female.

medication for at least 1 month before entering (A) as well as throughout the entire follow-up. Thereafter, they underwent dietary counseling by a dietitian once a week. Four weeks (B) after dietary intervention (7506 kJ-diet) as well as after another 4 weeks (C) after prickly pear ingestion (625 kJ, 50% from fibers and 50% from carbohydrates) were replaced by broiled edible pulp of opuntia robusta (250 g/day) for 4 weeks. The diet provided to the patients was constant, weighed and with the same energy amount through the entire study. Food records were collected controlling the macronutrient, energy and dietary fiber intake. Determination of lipids, lipoproteins, total cholesterol and triglycerides was determined by means of full enzymatic methods. Internal and external quality control was performed.

Routine safety parameters (GOT, GPT, γGT, alkaline phosphatase, CK) were determined by routine laboratory methods.

Isoprostane determination in plasma

Blood samples were anticoagulated with 2% EDTA and 1 mg/ml (final blood volume) acetylsalicylic acid (ASA). Immediate centrifugation at 4°C to obtain plasma was done at $1000 \times g$ for 10 minutes. Plasma was removed and stored at $\leq$70°C for not longer than 2 weeks until determination was made as described.[5] Samples were handled and processed as described.[6] The interassay variability was $5.5 \pm 1.7\%$, the intraassay variability $2.5 \pm 0.7\%$. Normal value: <20 pg/ml.

Serum 8-epi-PGF$_{2\alpha}$

Blood was drawn into glass vials. Vials were placed immediately into a water bath at 37°C for exactly 60 minutes. Serum was removed after centrifugation (4°C, $1000 \times g$, 10 minutes) and stored until determination was made (no longer than 2 weeks at < -70°C) as described.[7] The interassay variability was $3.8 \pm 1.2\%$, the intraassay variability was $1.9 \pm 0.7\%$. Normal value: 150–250 pg/ml.

Urinary 8-epi-PGF$_{2\alpha}$

Urine was collected over a period of 24 hours. 10 ml aliquots were adjusted to pH 4.0 with formic acid and taken for extraction. The eluate was subjected to silicic acid chromatography and further eluted. This final eluate was dried, recovered in buffer and assayed after dilution. Cross reactivity of the antibody with PGs was $<2\%$. Values are given in pg 8-epi-PGF$_{2\alpha}$/mg creatinine. The interassay variability was $6.4 \pm 2.3\%$, the intraassay variability $2.7 \pm 0.8\%$. Normal values: 150–250 pg/mg creatinine.

Statistical analysis

Data are presented as $\bar{x} \pm SD$; statistical analysis was performed by graph Pad Prism;[8] a $P < 0.01$ was considered as significant.

RESULTS

The individual prevalues of lipids, lipoproteins and isoprostanes are given in Table 2. Apart from height,

weight, body fat composition and HDL-values there was no difference in pre-study values between the sexes (Tables 1, 3, 4). The dietary counseling in the already ideal weight population of patients suffering from FH did not show any effect on either of the lipids or lipoprotein parameters. The regular ingestion of edible pulp of prickly pear did not show any influence on body weight, body mass index or body fat content. Total cholesterol, HDL- and LDL-cholesterol as well as triglycerides did not change after the dietary run-in period. Total cholesterol and LDL-cholesterol were significantly reduced by the ingestion of prickly pear, whereas no significant influence on HDL and triglycerides could be detected (Table 5). Lipid and lipoprotein response was more pronounced in females as compared to males (Tables 3, *6*).

8-epi-$PGF_{2\alpha}$ values in all the compartments tested were significantly higher as compared to healthy age-matched controls. Dietary counseling had no effect on plasma, serum and urinary isoprostane values either (Tables 3, *6*, *7*). Prickly pear addition resulted in a significant decrease in isoprostanes which was most pronounced in urine, followed by serum and plasma values. Interestingly, the responses on prickly pear ingestion in all compartments were more significantly pronounced in females as compared to males (Tables 3, *6*). There was a positive association of 8-epi-$PGF_{2\alpha}$ with total cholesterol ($r=0.4545$) (Figure 1), LDL-cholesterol ($r=0.4553$) (Figure 2) and a negative one with HDL ($r=-0.474$). In females the respective correlations were closer ($r=0.6774$, 0.6488 and -0.6932, respectively) (Figure 3). Throughout the whole study no influence on any of the safety parameters determined was detected.

DISCUSSION

The role of traditional food and lifestyle for health in various regions of the world is well documented.[9] The importance to medicine and nutrition of the qualitative and quantitative effects of traditional nutrients has increased enormously during the last decade. A variety of dietary compounds have been shown to exert beneficial effects on lipid peroxidation.[4,10] Locally-growing prickly pear, which is served in various forms, is used in the traditional medicine of native Americans,

Table 2 Lipids, lipoproteins and 8-epi-$PGF_{2\alpha}$ values in plasma, serum and urine in patients with FH

Patient No	CH	HDL	LDL	TG	P	S	U
1	284	46	216	86	21.4	306	329
2	306	52	231	104	27.6	284	345
3	275	50	200	91	22.3	291	336
4	293	51	216	137	25.4	308	341
5	308	43	244	82	33.6	327	389
6	338	47	251	157	28.5	296	362
7	271	51	196	96	27.0	306	356
8	267	44	195	122	29.1	299	340
9	295	34	226	154	30.2	317	351
10	336	40	258	161	26.8	288	359
11	351	47	277	127	32.7	305	367
12	324	44	260	69	28.6	295	352
13	257	40	196	93	25.3	291	340
14	288	50	208	117	29.7	302	381
15	294	43	221	98	30.2	315	390
$\bar{x} \pm$ SD	288 ± 28	45 ± 5	226 ± 26	112.9 ± 28.2	27.9 ± 3.3	302.0 ± 11.4	355.9 ± 18.4

CH: cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TG: triglycerides; P: plasma; S: serum; U: urine.

Table 3 Biological response on prickly pear intake in females

Parameter	A	B	C	A vs B	B vs C	A vs C
Body weight (kg)	62.8 ± 2.8	62.6 ± 2.4	62.7 ± 2.5	n.s.	n.s.	n.s.
Fat (%)	25.5 ± 1.6	25.4 ± 1.6	25.2 ± 1.6	n.s.	n.s.	n.s.
CH	300.9 ± 30.3	295.8 ± 25.7	278.9 ± 23.0	n.s.	n.s.	0.07
LDL	222.0 ± 34.6	222.5 ± 34.6	207.6 ± 24.6	n.s.	n.s.	0.04
HDL	47.0 ± 3.2	47.4 ± 3.7	48.0 ± 2.7	n.s.	n.s.	n.s.
TG	114.5 ± 26.2	114.8 ± 18.7	98.6 ± 24.1	n.s.	n.s.	0.07
IP plasma	27.9 ± 2.9	27.5 ± 2.6	25.3 ± 3.0	n.s.	n.s.	0.09
IP serum	300.3 ± 5.6	298.5 ± 6.4	284.8 ± 11.3	ns	0.007	0.002
IP urine	345.4 ± 14.4	347.4 ± 14.1	322.8 ± 12.4	n.s.	0.001	0.0003

IP: isoprostane; A: entry values; B: after dietary counseling; C: after prickly pear ingestion; n.s.: not significant; for units see Patients and Methods; for other abbreviations see Tables 1, 2.

Table 4 Sex differences in patients' characteristics, lipids, lipoproteins and isoprostane at study entry

	Females	Males	Significance
Age (a)	31.6 ± 6.3	35.0 ± 6.3	n.s.
Range	24–45	26–42	n.s.
Height (cm)	168.3 ± 3.5	180.1 ± 4.5	0.00006
Weight (kg)	62.8 ± 2.8	79.7 ± 3.7	0.0000001
Fat (%)	25.5 ± 1.6	22.1 ± 1.6	0.001
Disease duration (a)	3.3 ± 1.7	3.1 ± 1.0	n.s.
CH	300.9 ± 30.3	297.1 ± 22.4	n.s.
HDL	47.0 ± 3.2	42.0 ± 5.5	0.03
LDL	222.0 ± 34.6	227.4 ± 18.5	n.s.
TG	114.5 ± 26.2	111.1 ± 30.1	n.s.
IP plasma	27.5 ± 2.6	27.6 ± 2.6	n.s.
IP serum	300.3 ± 5.6	304.0 ± 15.3	n.s.
IP urine	354.4 ± 14.4	357.6 ± 29.9	n.s.
n	8	7	n.s.

Values are $\bar{x} \pm$ SD; (a): years.

Table 5 Influence of prickly pear intake on lipids, lipoproteins and 8-epi-$PGF_{2\alpha}$ in the total group of patients ($n = 15$; 8 f, 7 m)

Parameter	A	B	C	A vs B	B vs C	A vs C
Body weight (kg)	70.7 ± 9.0	70.4 ± 8.9	70.6 ± 9.0	n.s.	n.s.	n.s.
Fat (%)	23.9 ± 2.3	23.8 ± 2.3	23.7 ± 2.3	n.s.	n.s.	n.s.
CH	276.5 ± 22.0	293.3 ± 22.4	299.1 ± 27.0	n.s.	0.02	0.01
LDL	224.5 ± 28.4	223.0 ± 23.6	208.0 ± 20.3	n.s.	0.04	0.04
HDL	44.7 ± 5.1	44.9 ± 5.1	45.5 ± 4.9	n.s.	n.s.	n.s.
TG	112.9 ± 28.2	110.0 ± 23.0	100.4 ± 21.7	n.s.	n.s.	n.s.
IP plasma	27.9 ± 3.3	27.6 ± 3.0	25.6 ± 3.2	n.s.	0.05	0.03
IP serum	302.0 ± 11.4	299.3 ± 8.8	283.2 ± 14.5	n.s.	0.0007	0.0003
IP urine	355.9 ± 18.4	349.7 ± 17.3	323.9 ± 16.6	n.s.	0.0002	0.00002

For abbreviations see Table 3.

Table 6 Effects of regular prickly pear consumption on the various parameters in males

Parameter	A	B	C	A vs B	B vs C	A vs C
Body weight (kg)	79.7 ± 3.7	79.3 ± 3.6	79.6 ± 3.6	n.s.	n.s.	n.s.
Fat (%)	22.1 ± 1.6	22.0 ± 1.7	22.0 ± 1.7	n.s.	n.s.	n.s.
CH	297.1 ± 22.4	290.6 ± 13.5	273.9 ± 20.6	n.s.	0.07	0.04
LDL	227.4 ± 18.5	223.6 ± 15.9	208.4 ± 13.9	n.s.	0.05	0.03
HDL	42.0 ± 5.5	42.0 ± 5.0	42.6 ± 5.2	n.s.	n.s.	n.s.
TG	111.1 ± 30.1	108.0 ± 27.0	98.6 ± 24.1	n.s.	n.s.	n.s.
IP plasma	27.9 ± 3.7	27.6 ± 3.5	25.7 ± 3.4	n.s.	n.s.	n.s.
IP serum	304.0 ± 15.3	300.1 ± 10.8	281.4 ± 17.3	n.s.	0.02	0.01
IP urine	357.6 ± 21.9	352.3 ± 20.1	325.3 ± 20.2	n.s.	0.01	0.01

Abbreviations as in Table 3.

particularly in the Southern Arizona area and the neighbouring parts of Northern Mexico. For a long time this nutritional intervention has been demonstrated to show hypoglycemic[1] and hypolipidemic[2,11] actions, the latter having even been proven at the LDL-receptor level.[11,12]

An influence on oxidation injury, however, either on lipoproteins or in general has not been assessed yet. Isoprostanes have recently been identified to be reliable markers of in-vivo oxidation injury.[4,13] They are found in increased amounts in human arterial lesions,[6] in particular in foam cells. Our findings indicate that even in an ideal weight group of patients suffering from FH there is a significant improvement after prickly pear ingestion on in-vivo oxidation injury. In our group of patients (data not shown) normal fasting glucose values did not change. Furthermore, we were recently able to show that regular prickly pear intake results in an improved platelet function.[14] It is of special interest to note that the response of lipids and lipoproteins to regular prickly pear ingestion was better in males, while the decrease of 8-epi-$PGF_{2\alpha}$ in all the compartments was significantly better in females. Recently, a better response to statin therapy was discovered in females as compared to males. Although the exact fiber content (about 5%) of nopal is not available, it contains considerable amounts of cellulose and pectin varying among species.[15] The

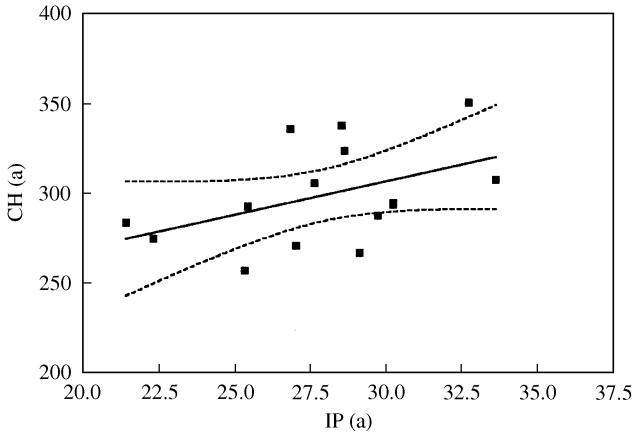

Fig. 1 Correlation between total cholesterol and 8-epi-$PGF_{2\alpha}$. There is a significant correlation between CH and IP in the total population.

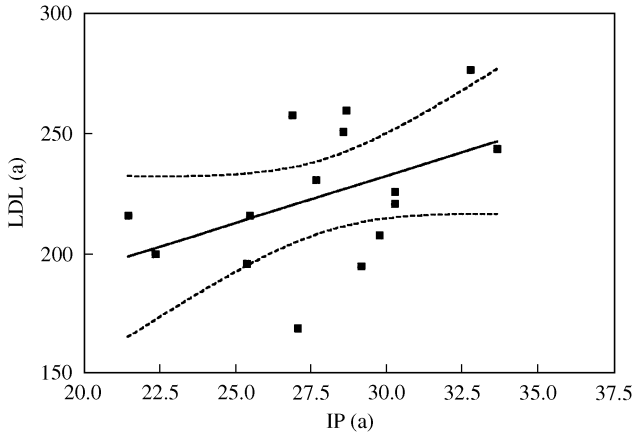

Fig. 2 Correlation between LDL and 8-epi-$PGF_{2\alpha}$. An even more close correlation exists between LDL and IP (total population).

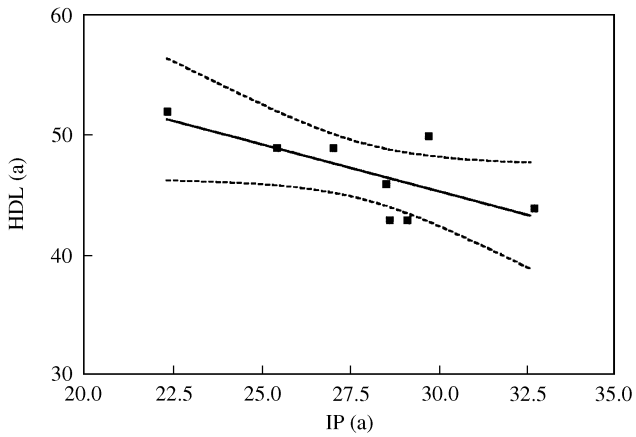

Fig. 3 Correlation between HDL and IP in females. There is a significant negative correlation between HDL and 8-epi-$PGF_{2\alpha}$.

underlying compound for the antioxidant effect of prickly pear remains unclear. The vitamin (A, B_1, B_2, C) content is insufficient to explain the benefit observed.[16] Developmental and seasonal changes in the consumption of prickly pear[17] may occur, however, being apparently small and thus not of significant biological value. An eventual influence of other cactus components such as the fruit on various parameters and mechanisms being of relevance in atherogenesis still needs to be determined.

CONCLUSION

According to our results prickly pear, besides the already known hypoglycemic, hypolipemic and antiplatelet effects, exerts a significant antioxidative action decreasing 8-epi-$PGF_{2\alpha}$ in plasma, serum and urine. Prickly pear might therefore be an interesting nutritional option to be used more widely as a (cheap) therapeutic coplayer even in patients with severe familial isolated hypercholesterolemia.

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