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Original

Prickly pear induces upregulation of liver LDL binding in familial heterozygous hypercholesterolemia

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Abstract

BACKGROUND: The hypoglycemic effect of prickly pear is well known by native local Indian population since a long time. Beside the beneficial effects on lipid metabolism, oxidation injury and platelet function has been claimed in experimental animals. We recently found an upregulation of apo-B/E receptor.
MATERIAL AND METHODS: We therefore examined 10 patients with isolated heterozygous familial hypercholesterolemia (FH) being enrolled in a dietary run-in phase of 6 weeks after dietary counselling and a further 6 weeks of prickly pear addition. Uptake of autologous ^{123}I-radiolabeled LDL was determined at entry as well as after 6 weeks of daily prickly pear ingestion.
RESULTS: We found a significant ($p < 0.0001$) increase in LDL--uptake by the liver (24.5 ± 4.9 vs. 31.1 ± 5.2%) and an enhanced decay in circulating blood. Total (298.0 → 268.0 mg/dl; $p < 0.0001$) and LDL-cholesterol (210.5 → 176.4 mg/dl; $p = 0.0001$) were significantly affected, while HDL ($p = 0.0629$) and triglycerides were not.
CONCLUSIONS: These findings demonstrate a significant upregulation of ^{123}I-LDL binding by prickly pear in FH-patients invivo and indicate that prickly pear exerts a significant hypolipidemic action via receptor upregulation.
Key words: prickly pear, LDL-receptor, familial hypercholesterolemia, ^{123}I-LDL-labeling

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Introduction

Pima Indians are showing an extremely high prevalence of impaired glucose metabolism [1] at already very young age. Asking the local witch doctors, opuntia (cactus) in various forms since long has been used as a dietary nutrient for treatment [2]. There are a number of experimental [3] and clinical [4, 5] reports indicating that prickly pear improves glucose tolerance and reduces glucose levels [6]. In diabetics reduction of blood glucose almost to the half has been reported [7–10], while in healthy people no such an effect has been described [6]. However, even in volunteers glucose tolerance after prickly pear ingestion improved [11, 12] most likely due to an increase in insulin sensitivity. Subsequently, nopal capsules have been successfully examined [13]. Examining the effect of prickly pear in non-diabetics with hyperlipidemia we found not only an improved glucose metabolism, but also significant changes in lipid metabolism [14].

Animal studies show that prickly pear pectin is able to decrease LDL-cholesterol, an effect which has been studied in guinea pigs to be due to an upregulation in apo-B,E receptor in the liver [15, 16]. No studies on human lipoprotein metabolism and in particular at the receptor level are available yet. To prove the benefit, in this paper we describe that regular daily prickly pear intake significantly improves LDL-receptor regulation.

Material and methods

Ten patients with recently discovered FH (for patients characteristics see Table 1) have been investigated. Except FH they were without any risk factor for the development of atherosclerosis and did not take any medication since at least 8 weeks prior to investigation.

Table 1. Patients characteristics

No.	Initials	Sex (m/f)	Age (years)	Height [cm]	Weight [kg]	Body fat (%)
1	CP	m	31	183	80	19
2	BS	f	27	174	66	22
3	HS	m	46	181	80	18
4	CD	m	39	176	79	23
5	SG	f	25	166	60	26
6	BP	m	27	177	74	16
7	HG	f	36	157	49	20
8	AW	f	40	165	58	21
9	HF	m	37	177	80	22
10	OW	m	43	190	82	17

m — males; f — females

Nutrition

The patients underwent dietary counseling by a dietitian once a week. 4 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. Body fat was determined by means of Tanita TBF-511.

LDL-isolation

For the isolation of human LDL 18 mL blood from overnight fasting normolipemics and from patients with FH were drawn into 2 Monovette vials (Sarstedt©, Germany) and anticoagulated 1:10 with 3.8% sodium citrate. LDL were separated by immunoaffinity chromatography.

Polyclonal anti-apo-B-antibodies were obtained by immunizing sheep with pure LDL. Gamma-globulins were precipitated from sheep plasma with ammonium sulfate (390 g/L; Sanabo©, Vienna, Austria) to a final concentration of 35% and further purified by immunoaffinity chromatography. For this purpose 3 g of pure LDL were coupled to 400 mL of BrCN activated Sepharose Cl 4B using a standard technique. The immunopurified antibodies were themselves coupled to BrCN-activated Sepharose Cl 4B and this support was used to isolate autologous apo B-containing lipoproteins (LDL, VLDL) from patients´ plasma: 10 mL of anti-LDL--sepharose Cl 4B gel were filled into a glass column (220 × 20 mm). The gel was extensively washed with 500 mL of isotonic NaCl-solution. 10 mL of patients´ citrated plasma were recirculated for 30 minutes over the column at a flow of 10 mL/min. The column was then washed with isotonic saline solution until it was protein-free (ε 260/280 nm $<$ 0.002). Lipoproteins were desorbed from the column with two bed volumes of 0.2 M glycine/HCl, pH 3.0, and dialyzed over night against 5 L of isotonic saline. The solution was then concentrated by ultrafiltration on AMICON XM 100 filters until a final concentration of 10 mg LDL/mL was achieved. Cholesterol in this preparation was measured by CHOD-PAP method (Boehringer Mannheim, Germany). This lipoprotein preparation was then ready for radiolabeling. Lipid electrophoresis showed pre-ß and ß-lipoproteins. Apoprotein concentration of the final solution as determined by radial immunodiffusion techniques showed absence of apo A-I, presence of apo B, apo CII, CIII and small amounts of apo E.

Radiolabeling of purified LDL [17]

An iodine monochloride (ICl)-stock solution (34 μmol/mL 6 M HCl) was purified before labeling by 3 extractions with $CHCl_3$ and diluted 1:100 with 2 M NaCl. To a microvial kept at 4°C approximately 1 mg purified lipoproteins, 1 M glycine buffer pH 10, about 1 mCi ^{123}I-NaI and freshly diluted ICl-solution were added to give a molar ratio ICl/apoprotein of 10/1. The reaction mixture (0.5–1 mL) was slowly stirred for 10 minutes at 4°C and sterile filtered into a dialysis bag which was kept in dialysis buffer (0.15 M NaCl, 0.01 M PO_4, pH 7.5, 0.2 mM EDTA) at 4°C until application for in-vivo studies. Immediately before this, it was filtered for the sterilization (Millipore sterile pyrogen-free filters, with a pore size of 0.2 μm). Radiochemical purity was determined by a) TCA-precipitation; b) electrophoresis on paper and polyacrylamid gel electrophoresis. Modification of the lipoprotein during processing and labeling was excluded by measuring TBARS, electrophoretic mobility and the isoprostane 8-epi-$PGF_{2\alpha}$ (via enzyme immunoassay).

The binding of the radiolabel was determined 1, 6, 12 and 24 hours after radiolabeling via the recovery and the lipoprotein fraction the radiolabel was bound to.

Gamma camera imaging

Patients underwent repeated thyroid gland blockade (before and the first 2 days after reinjection). Serial imaging (a total number of 60) for 30 minutes at frames of 30 seconds duration each (matrix size 64 × 64) was done under a LFOV-camera (Siemens©, Erlangen, Germany) in a region covering in antero-posterior view the liver, heart and lung. Immediately thereafter, whole body imaging with a speed of about 10 cm/min resulting in a total scanning time of about 20 minutes and SPECT imaging of liver to calculate liver volume under a double-headed gamma camera (Siemens©, Erlangen, Germany) were performed. Liver/heart and liver/lung activity ratios were calculated at different time intervals during the 30 minutes serial imaging, starting from one minute after the reinjection (necessary for ^{123}I-LDL equilibration in the blood). The areas used for the calculations over the regions of interest (ROI) remained constant during the first 60 serial images. The insertion of ROI over the liver during the whole-body scan allowed the quantification of the injected dose in the liver; this was then related to the patients´ liver volume.

The liver volume was estimated using transversal slices. In every slice a rectangular region was used. Within this region an isoconturic region at an activity level of 46% was automatically inserted [18]. The total pixel of this region is multiplied with the thickness of the slice and summed up for all the slices. Plasma decay of ^{123}I-LDL was determined from samples drawn during the initial 24 hours and subsequent counting.

Statistical analysis

Values are given in X ± SD; calculation for significance was performed by ANOVA. A $p < 0.01$ was considered significant.

Results

While dietary intervention in the 10 FH-patients did not reveal any significant change on lipids and lipoproteins, the addition of prickly pear resulted in a significant decrease in total- and LDL--cholesterol (Table 2), the extent being somewhat more pronounced in males as compared to females (Table 3). HDL-cholesterol exhibited no change after the dietary run-in phase ($p = 0.0629$). Total liver uptake (Table 4) in FH patients was 24.46 ± 4.96% and increased to 31.12 ± 5.23 ($p = 0.0001$). Subanalysis for females ($p = 0.0244$) and males ($p = 0.0016$) showed a somewhat more pronounced effect in the later. Prickly pear ingestion caused a significant rise in uptake. The recovery of ^{123}I-LDL was quite high (Table 5) during the initial 24 hours after reinjection indicating a rather stable tracer binding. Only minor amounts of the radiotracer were associated with other lipoproteins (HDL, VLDL). In addition, a significant shortage in half-life was monitored. No change in body weight and body fat content was monitored during the follow-up.

Table 2. CH-, LDL-CH and HDL-CH-values in patients at the different intervention periods

	CH			LDL-CH			HDL-CH		
	A	B	C	A	B	C	A	B	C
1	294	286	257	211	207	180	47	49	50
2	321	324	286	234	236	202	56	57	57
3	286	277	261	202	199	170	53	52	53
4	274	276	263	189	193	181	47	49	48
5	268	271	239	177	178	140	63	62	63
6	316	308	275	222	217	188	57	58	59
7	307	303	280	208	205	177	64	65	67
8	286	289	257	202	205	169	56	55	57
9	332	327	291	241	236	194	51	49	53
10	296	290	253	219	214	163	45	47	46
Total	298.0 ± 20.75	295.1 ± 19.72	268.0 ± 16.18	210.5 ± 19.50	210.2 ± 18.05	176.4 ± 17.43	53.9 ± 6.56	54.3 ± 6.13	55.3 ±6.62
Male	299.7 ± 20.99	294.0 ± 19.89	266.7 ± 14.05	214.0 ± 17.87	211.0 ± 15.19	179.3 ± 11.38	50.0 ± 4.52	50.67 ± 3.94	51.5±4.59
Female	295.3 ± 23.30	296.8 ± 22.40	270.0 ± 21.15	205.3 ± 23.40	209.0 ± 24.29	172.0 ± 25.55	59.75 ± 4.35	59.75 ± 4.57	61.0±4.90

A — before dietary counselling, B — after dietary intervention, C — after prickly pear ingestion, CH — cholesterol, LDL — low-density lipoprotein, HDL — high-density lipoprotein, ≤ p-values

Table 3. Statistical analysis (p-values) of data in Table 2

	CH			LDL-CH			HDL-CH		
	AB	AC	BC	AB	AC	BC	AB	AC	BC
Total	= 0.1049	< 0.0001	< 0.0001	= 0.8487	< 0.0001	< 0.0001	= 0.4226	= 0.0026	= 0.0629
Male	= 0.0184	= 0.0014	= 0.0014	= 0.0913	= 0.0035	= 0.0022	= 0.3939	= 0.0172	= 0.3165
Female	= 0.5266	= 0.0156	= 0.0146	= 0.1282	= 0.0001	< 0.0001	= 1.0000	= 0.1411	= 0.0796

Abbreviations see Table 2

Table 4. Liver-uptake and kinetics (T/2 in min) data in patients

No.	Sex	Liver uptake (%)		Kinetics [min]	
		B	C	B	C
1	Male	24.6	29.3	235	217
2	Female	17.6	25.3	242	222
3	Male	18.8	29.4	243	219
4	Male	26.2	35.6	206	181
5	Female	25.7	28.8	222	222
6	Male	31.6	43.2	217	193
7	Female	30.6	33.7	209	200
8	Female	25.4	29.8	230	221
9	Male	17.8	26.1	247	230
10	Male	26.3	30.0	229	216
Total	*	24.46 ± 4.95[1]	31.12 ± 5.23	228.0 ± 14.29[4]	212.1 ± 15.5
Male		24.22 ± 5.17[2]	32.27 ± 6.18	229.5 ± 15.67[4]	209.0 ± 18.4
Female		24.83 ± 5.37[3]	29.40 ± 3.46	225.8 ± 13.88[5]	216.3 ± 10.84

Abbreviations see Table 2; *$p = 0.0001$, [1]$p = 0.0001$, [2]$p = 0.0016$, [3] $p = 0.0244$, [4] $p = 0.0002$, [5] $p = 0.1030$

Table 5. Recovery (REC) and lipoprotein bound tracer in % over time

Time	1 h	6 h	12 h	24 h
REC	96 ± 2	87 ± 4	85 ± 6	84 ± 6
LDL	90 ± 2	80 ± 3	78 ± 2	76 ± 5
HDL	6 ± 2	6 ± 1	4 ± 2	6 ± 2
VLDL	0	1 ± 1	3 ± 1	2 ± 1

Data in x ± SD; the overwhelming amount of ^{123}I stays bound to the LDL-fraction throughout the initial 24 hours after reinjection

Discussion

Hypercholesterolemia is the major risk factor for atherosclerosis. Defective LDL-receptor binding by the liver is known as the key determinant [19]. *In-vivo* imaging of LDL-receptor binding to the liver [20] has been shown with scintiscanning using ^{99m}Tc [3], ^{111}In [21]- or ^{123}I [22]-labeling to be a reliable indicator for drug monitoring [18]. Using this approach this is the first report on an in-vivo upregulation of apo-B/E-binding by the liver in men after a dietary intervention. Regular daily intake of prickly pear results in a significant upregulation at the LDL-receptor level in patients suffering from FH. In parallel, LDL decreases. Fernandez et al. [16] found that prickly pear pectin intake decreases LDL cholesterol by increasing hepatic apo-B/E receptor expression in guinea pigs fed a hypercholesterolemic diet. However, no effect on cholesterol absorption was discovered. Hepatic apo-B/E receptor expression (Bmax) was increased by about 60%; similarly, the fractional catabolic rate was about 190% higher [15]. In our study the *in-vivo* relevance of increased LDL-liver binding is supported by an enhanced ^{123}I-LDL disappearance from blood. While prickly pear had no effect on hepatic microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase levels, ^{125}I-LDL binding to hepatic membranes was increased 1.7-fold, with the receptor affinity (Kd) being unchanged, but the receptor number (Bmax) being significantly enhanced [22]. Data on liver/heart ratio and liver/lung ratio strongly are supporting the increased ^{123}I-LDL-uptake by the liver. The stimulatory effect may also be to a minor part due to a change in eicosanoid profile, which in turn has been found to stimulate mRNA for the receptor protein [23]. The rather stable association of the label with the target protein indicates that dejodination is not occurring to a relevant extent.

The effect of compositional changes of the cactus during growth [24] and eventual seasonal alterations [25] or regional differences on LDL-binding has not been assessed yet.

Conclusions

These results provide evidence, that regular prickly pear ingestion induces a significant improvement of lipids and lipoproteins at the receptor level. These findings add a further piece of evidence on the beneficial actions of prickly pear, which already has been shown to improve platelet function [26] and oxidation injury [27] besides the well known hypoglycemic and lipid-lowering capacity.

References

1. Ravussin E, Valencia ME, Esparza J, Bennett PH, Schulz LO. Effects of a traditional lifestyle on obesity in Pima Indians. Diabetes Care 1994; 17: 1067–1074.
2. Perez RN, Ocegueda A, Munoz JL, Avila JG, Morrow WW. A study of the hypoglycemic effect of some Mexican plants. J Ethnopharmacol 1984; 12: 253–262.
3. Valllabhajosula S, Goldsmith SJ, Paidi M, Weihrauch ML, Parkter TM, Donelly T, Lpiszy H, Ginsberg HN. Non-invasive imaging of hepatic low-density lipoprotein (LDL) receptor activity in vivo with Tc-99m-LDL. J Nucl Med 1987; 28: A223.
4. Frati AC, Gordillo BE, Altamirano P, Ariza CR, Cortes Franco R, Chavez Negrete A. Acute hypoglycemic effect of opuntia streptacantha lemaire in NIDDM. Diabetes Care 1990; 13: 455–456.
5. Meckes Lozyoa M, Roman Ramos R. Opuntia streptacantha: a coadjutor in the treatment of diabetes mellitus. Am J Clin Med 1986; 14: 116–118.
6. Frati AC, Xilotl Diaz N, Altamirano P, Ariza R, Lopez Ledesma R. The effect of two sequential doses of opuntia streptacantha upon glycemia. Arch Invest Med Mex 1991; 22: 333–336.
7. Frati Munari AC, Yever Garces A, Islas Andrade S, Ariza Andraca CR, Chavez Negrete A. Studies on the mechanism of "hypoglycemic" effect of nopal (opuntia sp.). Arch Invest Med Mex 1987; 18: 7–12.
8. Frati Munari AC, Gordillo BE, Altamirano P, Ariza CR. Hypoglycemic effect of opuntia streptacantha lemaire in NIDDM. Diabetes Care 1988; 11: 63–66.
9. Frati Munari AC, Altamirano Bustamante E, Rodriguez Barcenas N, Ariza Andraca R, Lopez Ledesma R. Hypoglycemic action of opuntia streptacantha lemaire: study using raw extracts. Arch Invest Med Mex 1989; 20: 321–325.
10. Frati Munari AC, Rios Gil U, Ariza Andraca CR, Islas Andrade S, Lopez Ledesma R. Duration of the hypoglycemic action of opuntia streptacantha Lem. Arch Invest Med Mex 1989; 20: 297–300.
11. Frati Munari AC, Quiroz Lazaro JL, Altamirano Bustamente P, Banales Ham M, Islas Andrade S, Ariza Andraca CR. The effect of various doses of nopal (opuntia streptacantha lemaire) on the glucose tolerance test in healthy individuals. Arch Invest Med Mex 1988; 19: 143–148.
12. Frati Munari AC, de Leon C, Ariza Andraca R, Banales Ham MB, Lopez Ledesma R, Lozoya X. Effect of dehydrated extract of nopal (opuntia ficus indica mill) on blood glucose. Arch Invest Med Mex 1989; 20: 211–216.
13. Frati Munari AC, Vera Lastra O, Ariza Andraca CR. Evaluation of nopal capsules in diabetes mellitus. Caceta Med Mex 1992; 128: 431–436.
14. Wolfram RM, Kritz H, Schmid P, Efthimiou Y, Stamatopoulos Y, Sinzinger H. Effect of prickly pear (opuntia robusta) on glucose- and lipid-metabolism in non-diabetics with hyperlipidemia. Wr Klin Wschr 2002; 114: 840–846.
15. Fernandez ML, Lin EC, Trejo A, McNamara DJ. Prickly pear (opuntia sp.) pectin reverses low density lipoprotein receptor suppression induced by a hypercholesterolemic diet in guinea pigs. J Nutr 1992; 122: 2330–2340.
16. Fernandez ML, Lin EC, Trejo A, McNamara DJ. Prickly pear (opuntia sp.) pectin alters hepatic cholesterol metabolism without affecting cholesterol absorption in guinea pigs fed a hypercholesterolemic diet. J Nutr 1994; 124: 817–824.
17. McFarlane AS. Efficient tracer labelling of proteins with iodine. Nature 1958; 183: 53–59.
18. Sinzinger H, Virgolini I, Lupattelli G, Rauscha F. Quantitative imaging of human liver LDL-receptor in vivo. In: Gotto AM, Smith LC (eds). Drugs Affecting Lipid Metabolism. Excerpta Medica, New York, 1990; 319–327.
19. Lupattelli G, Virgolini I, Rauscha F, Sinzinger H. Quantitative imaging of human liver low density lipoprotein (LDL)-receptor in vivo. in: Höfer R,

Bergmann H, Sinzinger H (eds). Radioaktive Isotope in Klinik und Forschung. Schattauer, Stuttgart 1991; pp. 148–150.

20. Goldstein JE, Brown MS. Regulation of low-density lipoprotein receptors: implications for pathogenesis and therapy of hypercholesterolemia and atherosclerosis. Circulation 1987; 76: 504–507.
21. Virgolini I, Angelberger P, Li SR, Koller F, Koller E, Sinzinger H. ^{111}In-labeled low-density lipoprotein (LDL) binds with higher affinity to the human liver as compared to ^{123}I labeled LDL. J Nucl Med 1991; 32: 2132–2138.
22. Fernandez ML, Trejo A, McNamara DJ. Pectin isolation from prickly pear (opuntia sp.) modifies low density lipoprotein metabolism in cholesterol-fed guinea pigs. J Nutr 1990; 120: 1283–1290.
23. Sinzinger H, Kritz H, Virgolini I, Schmid P, Rogatti W. Prostaglandin E1 increases binding of ^{123}I low-density lipoprotein to the human liver in vivo. Eur J Clin Pharmacol 1996; 49: 515–520.
24. Meckes Lozyoa M, Ibanez Camcho R. Hypoglycaemic activity of opuntia streptacantha throughout its annual cycle. Am J Clin Med 1989; 17: 221–224.
25. Rodriguez FA, Contwell M. Developmental changes in composition and quality of prickly pear cactus cladodes (nopalitos). Plant Foods Hum Nutr 1988; 38: 83–93.
26. Budinsky A, Efthimiou Y, Stamatopoulos Y, Oguogho A, Wolfram R, Sinzinger H. Daily prickly pear consumption improves platelet function. Prostagl Leukotr Essential Fatty Acids 2003 (in press).
27. Budinsky A, Wolfram R, Oguogho A, Efthimiou Y, Stamatopoulos Y, Sinzinger H. Regular ingestion of opuntia robusta lowers oxidation injury. Prostagl Leukotr Essential Fatty Acids 2001; 65: 45–50.