Prickly pear changes ^{111}indium–LDL and ^{111}indium–HDL platelet binding Correlating to improvement of platelet function in hypercholesterolemia

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Abstract

Prickly pear consumption has shown beneficial effects on lipid metabolism and platelet function. Platelet low–density lipoprotein (LDL) and high–density lipoprotein (HDL) binding plays a key role in regulating the cellular functional activity. We therefore examined the role of prickly pear consumption on ^{111}Indium (^{111}In)–LDL and ^{111}In–HDL platelet binding in familial hypercholesterolemia (FH). Low–density (LDL) and high–density lipoproteins (HDL) were isolated by ultracentrifugation and subsequently radiolabeled with ^{111}In. The in–vitro binding of the radiolabel onto platelets was investigated in 14 patients with heterozygous FH. One month of dietary intervention was followed by a 2–months consumption of prickly pear. Dietary intervention improved Bmax (^{111}In–LDL but not ^{111}In–HDL) and had no effect on platelet function. Consumption of prickly pear, in contrast, resulted in a significantly increased ^{111}In–LDL and ^{111}In–HDL–binding by platelets which was associated with a significant decrease in platelet activity as determined via ADP–induced platelet aggregation, ß–thromboglobulin and 11–dehydro–thromboxane B_2. These findings demonstrate that ^{111}In–LDL and ^{111}In–HDL specific binding sites on platelets which are lower in heterozygous FH can be upregulated by regular prickly pear consumption. This alteration is paralleled by a deactivation of platelets. The underlying mechanism still remains to be elucidated.

Keywords: LDL–binding, ^{111}Indium, radiolabeling, ß–thromboglobulin (ßTG), prickly pear, platelet function, ADP–induced aggregation.

Introduction

The regulation of LDL by receptors has been shown long ago to be a key determinant in lipid metabolism (Goldstein *et al.*, 1987). Impairment of LDL–binding influences LDL catabolism, subsequently regulating blood lipids. Specific binding sites have been identified on a variety of tissue and circulating cells (Curtis *et al.*, 1984; Weisgraber *et al.*, 1978). A mutation of the LDL receptor is known to cause familial hyperlipidemia. Patients with FH exhibit an activated platelet population as assessed by a great variety of different tests (Aviram *et al.*, 1982, Carvalho *et al.*, 1974a, Jäger *et al.*, 1982, Silberbauer *et al.*, 1977). Lipid lowering agents and to a lesser extent dietary intervention have been shown to at least partially normalize an activated platelet population (Carvalho *et al.*, 1974b). Patients with isolated hypercholesterolemia show an increased cholesterol and phospholipid content in the platelets (Shattil *et al.*, 1975). As the cellular cholesterol content is associated with platelet reactivity to stimuli any alteration may provide an explanation for activation of platelet function. A direct action through platelet LDL– and HDL–binding could be a key mechanism (Virgolini *et al.*, 1992a; 1992b). Since long, prickly pear has been used as a dietary nutrient for treatment of diabetes by Pima Indians (Ravussin *et al.*, 1994). The great variety of potentially active nutrients and their multifunctional properties (Stintzing *et al.*, 2005) make cactus pear a promising candidate for the production of health promoting food and food supplements (Feugang *et al.*, 2006). Experimental (Fernandez *et al.*, 1990; 1992; 1994) and clinical studies in men revealed that prickly pear among others improves blood lipids (Wolfram *et al.*, 2002), increases liver LDL–binding (Palumbo *et al.*, 2003) and improves platelet function (Wolfram *et al.*, 2003). Platelet lipoprotein binding has originally been reported for LDL by Hassal *et al*. (1990). We therefore examined, whether high affinity LDL– and HDL–binding of platelets and platelet function are affected in patients with FH during regular prickly pear consumption.

Patients and methods

We determined the binding of ^{111}Indium–LDL and ^{111}Indium–HDL to washed human platelets derived from 14 patients (nine m, five f; aged 24–44 years) newly diagnosed with heterozygous FH characterized at the receptor level before (and naïve to lipid lowering drugs so far) and after four weeks of dietary intervention and subsequently during two months of prickly pear (250 g/d) ingestion (for patients characteristics see Table 1). In parallel, platelet function (ADP–induced platelet aggregation, ß–thromboglobulin (ßTG) and plasma 11–dehydro–thromboxane (TX) B_2) was assessed. In all the patients lipoprotein (a) (Lp(a)) was below 30 mg/dl and thus in the normal range. FH patients taking drugs known to interfere with platelet function, lipid metabolism, or the prostaglandin (PG) system were excluded. All FH–patients were on a low–fat diet for at least 4 weeks, before the prevalue was drawn. The study was performed according to the Helsinki declaration. Participants were giving written informed consent.

Lipoprotein isolation

Lipoproteins were isolated from five healthy donors (m, 31–38 years) who had not received any medication since at least two weeks after a >16 hours overnight fasting. All normolipemic control subjects did not smoke and did not have any other known risk factor for the development of atherosclerosis. Binding studies for each patient were run with the isolated LDL and HDL, respectively, from the same volunteer. To examine individual variation of the binding data, the five healthy volunteers were investigated twice within three months before this study.

30 mls of blood were collected via siliconized needles into heparin–coated vials after an at least 16 hours overnight fast. In all the blood donors, normolipemia was assured by determination of total cholesterol, LDL cholesterol, HDL–cholesterol, triglycerides, Lp(a), apoA_1 and A_2 as well as apoB.

Table 1. Patients characteristics.

Patient	age	Sex	Height (cm)	Weight (kg)	CH	TG	HDL	Lp(a)
1	24	M	179	77	287	100	71	7
2	31	F	166	57	245	172	39	11
3	26	M	185	84	316	180	49	3
4	36	F	171	60	267	169	46	19
5	35	M	169	71	303	136	43	21
6	44	M	178	80	244	163	42	6
7	29	M	182	78	239	133	39	4
8	37	F	157	48	281	94	41	7
9	31	M	177	75	269	168	49	8
10	39	M	188	84	300	142	49	5
11	28	M	178	75	307	100	65	15
12	30	F	160	50	303	186	45	14
13	37	F	164	53	259	169	48	7
14	39	M	167	68	264	137	50	9
Mean	33.29	9/5	172.83	74.07	277.43	134.71	48.29	9.71
SD	–	–	–	–	25.76	31.09	9.24	15.55

x±SD; P= patient; CH= cholesterol; TG= thromboglobulin; HDL= high–density lipoprotein; Lp= lipoprotein

Lipoproteins were prepared from the fresh plasma by isopycnic ultracentrifugation (Havel *et al*., 1985) using potassium bromide for density adjustment as described previously (Folch *et al*., 1957). Briefly, after an 18–hours ultracentrifugation (L5–75 ultracentrifuge, Beckman Instruments, Inc., Palo Alto, CA; 40.3 Ti rotor at 40000 rpm and 10°C), the VLDL fraction was withdrawn and the pellet re–suspended in potassium bromide solution at a density of 1.063 g/ml, i.e. KBr (grams)=plasma volume (milliliters) x (1.063–1.019) x 0.94 (constant)/[1–(0.295 x 1.063)] and centrifuged against the density gradient for 18 hours at 40000 rpm and 10°C. The supernatant (d=1.019–1.063 g/ml) contained the LDL fraction. HDLs (d=1.063–1.21 g/ml) were isolated by further sequential ultracentrifugation (43,000 rpm; 44 hours; 10°C; KBr d=1.210 g/ml), i.e. KBr (grams)=plasma volume (milliliters) x (1.210–1.063) x 0.94 (constant)/[1–(0.297 x 1.210)]. The lipoproteins were dialyzed against phosphate–buffered saline (PBS) (pH 7.4), containing 0.1 mg/ml EDTA and stored at 4°C for not longer than one week. The total protein content of the lipoproteins was analyzed by the method of Lowry (Lowry *et al.*, 1951) and used to determine LDL amounts for labeling. Lipoproteins were concentrated by ultra–filtration using Centrisart UF membranes (Sartorius, Göttingen, Germany). The apoprotein composition of each of the lipoprotein classes was assessed by radial immunodiffusion.

Radiolabeling

For each series of experiments, LDL and HDL from one normolipemic subject were used for labeling with ^{111}In (Rosen *et al.*, 1990; Virgolini *et al.*, 1992a and 1992b). ^{111}In was chosen in view of the excellent in–vitro and in–vivo HDL– and LDL–binding data (showing advantages over ^{99m}Tc and ^{123}I). Briefly, to a microvial equipped with a magnetic stirrer, 1 mg LDL in 200 µl of 0.5 M $NaHCO_3$, and 36 µg cyclic diethylenetriaminepentaacetic acid (DTPA) anhydride (Sigma Chemical CO., St. Louis, MA) in 9 µl dry dimethyl sulfoxide (Merck, Darmstadt, Germany) were added. The mixture was slowly stirred for 1 hour and applied to a 5x40 mm Sephadex G50F column (Pharmacia, Uppsala, Sweden) equilibrated in metal–free acetate–buffered saline (ABS) (pH 5.5).

The column was eluted with ABS, and the protein fraction (240 μl) was collected into a microvial. To this mixture, 600 μl (^{111}In) Cl_3 in 40 μl of 0.04 M HCl was added under gentle mixing. After 1 hour at 22°C, the reaction mixture was applied onto a second ABS–equilibrated Sephadex G50F column. The ^{111}In–labeled protein fraction (350 μl) was collected and mixed with one mM DTPA in PBS to give 1 ml of the final product solution. The integrity of protein was tested by trichloroacetic acid (TCA) precipitation (10% TCA final concentration). The amount of radioactivity localized in the lipid moiety was 5–10% (of the total incorporated radioactivity) as estimated by extraction with chloroform/methanol (Folch *et al.*, 1957).

Radiochemical purity was determined by 1) thinlayer chromatography (TLC) with silica gel plates (Merck) and an eluent composed of methanol:10% ammonium formate:0.5 M citric acid (20:20:10 vol/vol/vol), 2) cellulose acetate electrophoresis with 0.05 M barbital buffer (pH 8.6), containing 1 mM EDTA and 1% human serum albumin at 300 V for 20 minutes, and 3) polyacrylamide gel electrophoresis (PAGE) with gradient gels (8–18%) and a gel buffer of 0.12 M tris(hydroxymethyl)aminomethane (Tris), 0.12 M acetate, and 0.1% sodium dodecyl sulfate (pH 6.4) (LKB/Pharmacia, Uppsala, Sweden), at 50 mA/gel, 200–600 V for 60 minutes.

Binding studies

The effect of a 1–month dietary intervention on LDL and HDL binding sites was studied. For this period, each patient followed a dietary protocol for a daily total fat (35%) and cholesterol intake of <300 mg/d. The nutritional behaviour was monitored by a dietician. LDL and HDL binding sites were estimated before and after a four–weeks dietary treatment as well as after eight weeks daily ingestion of prickly pear.

Platelet isolation and characterization

Blood processing was performed using plastic ware at 22°C. Venous blood (50 ml) was collected through siliconized needles into 3x20 ml plastic syringes containing acid–citrate–dextrose (3:7, vol/vol). Platelet rich plasma (PRP) was prepared by centrifugation (100g for 10 minutes). In a further centrifugation step (1000 g for 25 minutes), a platelet pellet was obtained and washed twice in buffer containing 0.9% NaCl, 50 mM Tris HCl, and 2% Na–EDTA (20:1:2 vol/vol/vol, pH 7.2). The washed platelet pellet was taken up in assay buffer containing 50 mM Tris–HCl (pH 7.5), 5 mM $CaCl_2$, and 5 mM $MgCl_2$. Platelet count was determined electronically and by counter flow.

The effectiveness of the washing procedure in removing autologous plasma proteins was assessed by adding ^{111}In–LDL of ^{111}In–HDL, respectively to the PRP. Based on the recovery of radioactivity in the isolated platelet suspension, 7±1 ng LDL (3±2 ng HDL) were present per 10^9 platelets.

Residual binding of autologous LDL to washed platelets was also investigated directly with anti–apoB antiserum. Washed platelets (2.10^7) were lysed in 2% sodium dodecyl sulfate buffer and electrophoresis performed on a Pharmacia gradient gel (2–16%). After blotting onto nitrocellulose membranes, the blotted proteins were probed by anti–apoB antiserum (Behring AG, Vienna, Austria) and developed by peroxidase–conjugated anti–rabbit antibody and 4–chloro–1–naphtol. By this method, LDL protein is detectable in the range of 50–200 pg per band. No apoB was found by this method. Repeated testing of volunteer LDL–binding revealed no significant variation (< 4%).

ADP–induced platelet aggregation

Platelet aggregation (anticoagulation with 1/9 vol 3.8 % sodium citrate) was studied in a Born–type (Born, 1962) aggregometer. Platelets ($2.5x10^5$/μl in 600 μl) were aggregated with 1 μM ADP (100 μl). The aggregation response was assessed by the height (T_{max}) and slope (α) of the response curve.

ß–thromboglobulin
Blood from non–occluded veins was carefully collected into ß–TG sampling tubes under the addition of PGI_2 to avoid artificial release. After preparation of platelet poor plasma (PPP) at 4°C the samples were frozen at –20°C until examination. The concentration of ß–TG was determined using the commercially available RIA–kits (Abbott, North–Chicago, USA; Amersham International, Buckinghamshire, UK, respectively). The inter–assay variation was 4.9±1.7%, the intra–assay variation 3.7 ± 1.2 %.

11–dehydro–TXB_2
Coagulation of blood was prevented with 2% sodium EDTA under the addition of 100 mg/ml acetylsalicylic acid (ASA) for cyclo–oxygenase inhibition. Samples were centrifuged at 4°C and 1500xg for 15 minutes. Plasma was stored for no longer than 2 weeks at –70°C. Specific RIA was performed according to Virgolini *et al*. (1988) in un–extracted samples using the double–antibody technique for separation of free and antibody bound ligand. The inter–assay variation was 5.6±1.4%, the intra–assay variation 3.9±1.1%.

Bindings Assays
To investigate ligand binding to platelets, direct binding experiments were carried out. Incubations were done in duplicate. Conditions for the assay system for ^{111}In–LDL and ^{111}In–HDL were essentially the same. The intra–assay variability was 3.6±0.8%; the inter–assay variability was 6.0±1.0% (LDL) and 6.6±1.1 (HDL). In parallel experiments the time course of association in binding was studied by incubating 5.10^6 platelets with ^{111}In–LDL (1.5 μg protein/ml) in the absence (total binding) and presence (non–saturable binding) of unlabeled LDL (100 μg protein/ml) for 1–120 minutes. Dissociation of binding was induced by the addition of an excess amount of unlabeled LDL (100 μg protein/ml) at different time intervals (1–60 minutes) at equilibrium.

For competition experiments, the cells were incubated at room temperature for 45 minutes with 5 μg protein/ml ^{111}In–LDL (925 cpm/ng protein) in the absence (total binding) and presence (non–saturable binding) of increasing concentrations (0.1–500 μg protein/ml) of unlabeled LDL.

In saturation experiments, the cells were incubated with increasing concentrations of ^{111}In–LDL (0.1–70 μg protein/ml) in the absence (total binding) and presence (non–saturable binding) of unlabeled LDL (100 μg protein per milliliter).

After incubation, the tubes were rapidly centrifuged (1500 g for 10 minutes at 4 °C) to separate free from membrane–bound radio–ligand. After washing twice, the pellet was counted in a gamma–counter for one minute.

Specific binding was determined as the difference of total and non–saturable binding. In typical experiments, non–saturable binding (determined in the presence of an excess of unlabeled LDL) amounted to <10% of total binding in the high–affinity ligand range: specific binding=total binding – non–saturable binding = 100–(<10) = >90.

In the absence of platelets, the application of 30 μg protein of ^{111}In–LDL resulted in the recovery of less than 1 μg protein of ^{111}In–LDL in the tip of the tube after centrifugation (<4%). This amount was identical for incubations of total and non–saturable binding.

Analysis
Binding data were calculated according to Scatchard (1949). Values are presented as mean± SD.

Nutrition

The patients underwent dietary counseling by a dietitian once a week. Four Weeks (B) after dietary intervention (7500 kJ–diet) as well as after another eight weeks (C) after prickly pear ingestion by an isocaloric diet were replaced by *Opuntia lindheimerii* fruit (250 g/day in which the epidermis was removed), for four weeks that were obtained from Greece (Figure 1). 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 done by means of full enzymatic methods. Internal and external quality control was performed. Safety parameters (liver: GOT, GPT, γGT and kidney function: creatinine) were monitored.

Figure 1. *Opuntia lindheimerii* plants with fruits growing in Greece from which the fruits used in these experiments were obtained.

Results

As compared to the pre–values lipid (cholesterol, triglycerides) and lipoprotein (HDL, LDL) did not change during the dietary run–in–phase significantly (Table 2). Prickly pear ingestion resulted in a decrease in total– ($p=0.0403$) and LDL–cholesterol ($p=0.0249$) (Table 2), but no change in triglycerides and HDL ($p = 0.0403$) occurred. Dietary intervention improved Bmax (^{111}In–LDL) but not ^{111}In–HDL and did not influence KD and IC_{50}. Prickly pear consumption over a period of 8 weeks significantly ($p<0.01$) increased ^{111}In–LDL–platelet binding (Table 3) but had no significant effect on Kd and IC_{50}. Similarly, a significant ($p<0.0094$) improvement in ^{111}In–HDL–platelet binding (Table 4) was seen, but again not in Kd and IC_{50}. Platelet function improvement (Table 5) was achieved by prickly pear consumption, but not by dietary intervention before. Comparable beneficial findings were obtained for all the three different platelet function parameters (i.e. ADP–induced platelet aggregation [$p=0.0025$], ß–TG [$p=0.0217$], 11–dehydro–thromboxane B_2 [$p=0.0108$]). The improved ^{111}In–LDL– and ^{111}In–HDL–binding is significantly correlated to improvement in platelet function ($p<0.01$). No change in any of the safety parameters was observed.

Table 2. Lipids and lipoproteins.

Patient	CH			TG			HDL			LDL			Lp(a)
	P	D	C	P	D	C	P	D	C	P	D	C	P
1	287	290	276	100	109	97	71	70	71	196	198	186	7
2	245	242	240	172	166	167	39	40	41	171	169	166	11
3	316	304	286	180	184	171	49	46	50	231	221	202	3
4	267	265	250	169	165	174	46	45	44	187	187	171	19
5	303	306	300	136	129	135	43	44	46	233	236	227	21
6	244	242	224	163	148	157	42	43	45	169	169	148	6
7	239	235	220	133	134	130	39	40	42	173	166	152	4
8	281	276	236	94	90	93	41	40	43	221	218	174	7
9	269	257	250	168	150	164	49	48	50	186	177	167	8
10	300	294	275	142	136	144	49	49	49	223	218	197	5
11	307	299	284	100	105	97	65	66	68	222	212	197	15
12	303	288	290	186	182	178	45	45	46	221	207	209	14
13	259	250	222	169	166	163	48	48	47	177	169	142	7
14	264	244	212	137	139	141	50	51	54	187	165	130	9
Mean	277.43	270.86	254.64	146.36	143.07	143.64	48.29	48.21	49.71	199.79	193.71	176.29	9.71
SD	25.76	25.84	29.94	31.09	28.59	29.80	9.24	9.08	9.10	24.11	24.79	28.00	5.55
P	–	n.s.	< 0.05	–	n.s.	n.s.	–	n.s.	n.s.	–	n.s.	< 0.05	

x ± SD; P= prevalue; D= dietary intervention; C= after prickly pear consumption; LDL= low–density lipoprotein; n.s.= not significant; further abbreviations see Table 1.

Table 3. ^{111}In–LDL–binding data.

Patient	Bmax			Kd			IC_{50}		
	P	D	C	P	D	C	P	D	C
1	391	434	725	6	6	7	13	13	14
2	546	627	916	11	11	13	18	17	16
3	597	643	751	12	13	13	24	25	26
4	588	616	936	7	7	6	20	20	21
5	694	728	1116	9	8	9	25	25	26
6	488	513	824	11	10	12	16	15	17
7	361	407	688	11	12	12	25	25	24
8	706	878	1012	8	7	9	17	16	18
9	601	479	897	8	7	8	20	20	21
10	543	581	831	9	9	10	19	18	19
11	737	744	991	10	9	9	20	19	20
12	581	608	1054	11	12	13	22	21	22
13	497	503	931	10	11	10	24	24	23
14	806	801	1147	12	13	15	24	23	25
Mean	581.14	611.57	915.64	9.64	9.64	10.43	20.50	20.07	20.86
SD	126.17	139.40	141.32	1.86	2.41	2.62	3.70	3.97	3.76
p	–	< 0.05	< 0.01	–	n.s.	n.s.	–	n.s.	n.s.

x ± SD; Bmax= maximal binding capacity; Kd= dissociation constant; IC_{50}= half–maximal association (µg protein/ml); other abbrevations as in Table 2.

Table 4. Platelet ^{111}In–HDL–receptor binding.

Patient	**Bmax**			**Kd**			**IC_{50}**		
	P	D	C	P	D	C	P	D	C
1	1324	1417	1612	6	7	6	20	19	18
2	1086	1124	1397	10	10	8	23	22	20
3	798	839	1116	14	14	12	27	25	21
4	971	1086	1312	9	9	8	22	22	20
5	1524	1608	1774	8	7	8	30	29	26
6	1208	1288	1436	7	8	6	25	23	20
7	1054	1148	1392	11	11	10	16	17	15
8	1726	1795	1933	12	11	10	12	13	13
9	884	952	1096	9	9	7	25	23	20
10	1186	1237	1549	11	10	9	31	27	25
11	1397	1486	1713	13	13	11	26	26	20
12	1421	1497	1633	12	11	9	19	20	17
13	986	1091	1347	14	15	13	28	29	25
14	1608	1699	1958	10	10	9	24	21	22
Mean	1226.71	1304.79	**1519.14**	10.43	10.36	9.50	23.43	22.57	20.14
SD	282.19	286.51	**269.04**	2.47	2.41	2.08	5.30	4.52	3.70
P	–	n.s.	**< 0.01**	–	n.s.	n.s.	–	n.s.	n.s.

Bmax (ng protein of ^{111}In–HDL bound/10^9 platelets); Kd= dissociation constant; IC_{50}= half–maximal association (µg protein/ml); for other abbreviations see Table 2.

Table 5. Platelet function parameters.

Patient	**ADP[1]**			**ßTG[2]**			**11–DH–TXB_2[3]**		
	P	**D**	**C**	**P**	**D**	**C**	**P**	**D**	**C**
1	67.6	68.3	60.3	42.4	44.0	39.6	32.2	33.1	27.6
2	62.7	63.1	55.6	31.5	36.6	30.2	29.8	29.5	26.0
3	73.5	72.8	66.9	45.8	46.7	42.7	35.7	36.2	30.8
4	68.2	68.2	63.0	45.2	45.5	40.6	36.9	36.4	32.6
5	70.7	69.5	63.8	44.7	43.7	39.7	39.7	38.8	35.2
6	65.0	64.4	60.0	42.2	43.8	37.8	35.6	35.5	30.8
7	76.2	75.2	69.7	48.6	46.9	45.3	40.8	40.0	34.9
8	64.4	64.1	57.4	40.2	38.7	39.0	39.3	38.7	36.0
9	63.7	60.2	58.3	41.4	40.9	36.2	37.6	33.5	27.7
10	64.0	62.5	55.1	42.3	41.8	40.3	34.5	33.9	33.5
11	67.7	68.0	63.2	42.0	42.5	42.0	38.2	38.5	36.8
12	60.3	59.5	52.4	37.8	38.4	32.6	34.6	35.1	29.2
13	56.9	55.3	50.2	35.0	36.3	34.7	37.5	39.4	38.0
14	65.6	63.8	57.4	40.7	39.9	35.8	45.7	44.0	39.9
Mean	66.19	66.35	**59.52**	41.41	41.84	**38.32**	37.01	36.61	**32.79**
SD	5.05	5.36	**5.45**	4.25	3.50	**4.09**	3.88	3.62	**4.25**
p	–	–	**< 0.005**	–	–	**< 0.05**	–	–	**< 0.02**

x ± SD; [1]values in % maximal aggregation; [2]values in ng/ml; [3]values in pg/ml; other abbreviations as in table 2.

Discussion

Pima Indians are well known for an extremely high prevalence of impaired glucose metabolism at already very young age (Ravussin *et al*., 1994). *Opuntia* spp. in various forms has been used since long due to the anti–diabetic action. The mechanisms underlying the proven benefit are still incompletely understood. Numerous studies indicate that prickly pear pectin is able to decrease LDL–cholesterol, an effect which has been shown in guinea pigs to be due to an up–regulation in apo–B,E receptor binding to the liver (Fernandez *et al*., 1992, 1994). A decrease in total– and LDL–cholesterol, but no change in HDL was observed (Wolfram *et al*., 2002) in human as well as after *Opuntia ficus–indica* (Ennouri *et al*., 2005) ingestion in rats. In mice, *O. ficus–indica* var. decreased significantly total cholesterol, LDL–cholesterol and triglycerides (Oh *et al*., 2006), HDL was not affected, too. Similar findings were obtained after administering on *Opuntia* powder, the benefits being more pronounced in hyperlipidemic vs. normolipimedic animals (Li *et al*., 2005). Earlier, we were able to show that in 10 patients with heterozygous FH the LDL–uptake by the liver in–vivo was significantly increased by 24% at mean. In parallel, total and LDL–cholesterol significantly decreased (Palumbo *et al*., 2003) after prickly pear consumption.

Interestingly, Fernandez *et al*. (1990) reporting an increase in hepatic apo–B, E receptor were unable to find an effect of prickly pear on cholesterol adsorption. Hepatic apo–B, E receptor expression (Bmax) was increased by about 60%, while the fractional catabolic rate was about 190 % higher. To the best of our knowledge no data on HDL– and LDL–binding to platelets are available. Beside changes in cellular membrane composition (increase in cholesterol and arachidonic acid) recognition of lipoproteins by specific platelet receptors regulates platelet activation, aggregation and release. Platelet LP receptors present different biochemical, immunological and genetic characteristics, compared to classical LDL–receptors (Pedreño *et al.*, 2000). While the protein fraction is of key importance in classical LDL–receptor recognition, the lipid fraction seems to have a more relevant role in platelet receptor recognition (Pedreño *et al.*, 2000). A variety of influences of LDL (and HDL) on functional response of platelets occur in the presence of abnormal plasma values.

While prickly pear showed no influence on hepatic microsomal 3–hydroxy–3–methylglutaryl co–enzyme A–reductase levels, the ^{125}I–LDL binding to hepatic membranes, was 1.7–fold increased, as well as was the number of receptors. The receptor affinity, however, remained unchanged. The stimulatory effect may be in part due to the change in eicosanoid profile which already has been shown to stimulate the messenger RNA for the receptor protein.

Pedreño *et al*. (1997) showed that the platelet LDL–receptor recognizes with the same affinity both native and oxidized LDL–particles. A strong relation between 8–epi–$PGF_{2\alpha}$ and fat intake (positively) and dietary antioxidants (negatively) in a group with metabolic syndrome was found (Sjogren *et al.*, 2005). The isoprostane 8–epi–$PGF_{2\alpha}$ being a sensitive measure for in–vivo oxidation injury itself exerts a mild proaggregatory action on platelets (Leitinger *et al.*, 1997). Similarly, we found a decrease in 8–epi–$PGF_{2\alpha}$ after prickly pear consumption (Budinsky *et al*., 2001) in a randomized, cross–over, double–blind treatment. In healthy human volunteers, *O. ficus–indica* reduced oxidative stress as assessed via plasma 8–epi–$PGF_{2\alpha}$ and malondialdehyde (Tesoriere *et al.*, 2004a). Similar findings were reported from a glycoprotein isolated from *O. ficus–indica* (Oh *et al*., 2006). This was discussed as an improved capacity to handle increased oxidative stress. Betalains have been discovered in cactus pears and found to be protective against in–vitro copper–induced oxidation of LDL (Tresoriere *et al.*, 2004b).

The natural antioxidant content of the cactus fruits was not analyzed, therefore not allowing correlating total phenolics and betalaine contents (Stintzing *et al.*, 2005) with the platelet lipoprotein binding capacity.

Simvastatin (20 mg) and atorvastatin (10 mg) have been shown to reduce platelet activity by exposure of CD36 and lectin–like ox–LDL receptor–1 (LOX–1) before significant reduction of LDL (Bruni *et al*., 2005). Specific ox–LDL–receptors (Pedreño *et al*., 1994) have not been determined in this investigation. Reduction of LOX–1 is considered a direct anti–atherosclerotic mechanism related to the role of oxLDL in platelet activation and platelet–endothelium interaction. It has been shown that oxLDL similar to native LDL enhances in–vitro induced platelet aggregation (Pedreño *et al.*, 1997). These our findings support the hypothesis that high–affinity lipoprotein binding sites on platelets are significantly related to their functional activity. Additional prickly pear consumption and platelet lipoprotein binding sites may thereby significantly contribute to a decreased platelet functional activity.

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