Discussion

These observations suggest either that small quantities of iron may stimulate the production of large quantities of apoferritin or that production of apoferritin may occur independently of iron. A serum ferritin concentration greater than 55 µg/l, however, would appear to exclude appreciable iron deficiency in the presence of synovial inflammation. The difficulties in assessing total body iron stores from bone-marrow values are highlighted by the observation that iron may be redistributed to other active reticuloendothelial organs (synovial membrane). Thus the much greater fluctuations in serum ferritin concentrations observed during changes in inflammatory activity⁸ probably reflect a fundamental disturbance in iron kinetics with a redistribution of iron from the plasma, red-cell, and bone-marrow compartments to any stimulated reticuloendothelial cell and not a non-specific production of apoferritin protein.

A teleological explanation for this phenomenon relates to the nutritional requirement of replicating bacteria for iron. The drop in serum iron concentration observed early during inflammation and infection may be seen as an attempt to deprive a potential pathogen of an essential growth factor. The need of reticulo-

endothelial cells to manufacture a suitable storage protein for this iron in advance is understandable in view of the known stimulatory effect of free iron on oxidative radical reactions.⁶

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Prostacyclin and thromboxane in diabetes

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Abstract

Concentrations of the stable antiaggregatory prostacyclin metabolite 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PGF $_{1\alpha}$) and of the proaggregatory thromboxane A_2 metabolite thromboxane B_2 were measured by radioimmunoassay in plasma from 53 diabetics. In 33 of these patients the ability of platelets to produce thromboxane B_2 during spontaneous clotting was also studied.

Plasma 6-keto-PGF $_{1\alpha}$ concentrations were higher ($p < 0.05$) in the diabetics (mean $107.7 \pm SE 7.6$ ng/l) than in non-diabetic controls matched for age and sex (87.5 ± 4.7 ng/l), and diabetics with microangiopathy ($n = 28$) had higher ($p < 0.01$) concentrations (124.3 ± 10.8 ng/l) than those without microangiopathy ($n = 25$; 89.2 ± 9.3 ng/l). Plasma thromboxane B_2 concentrations were also higher ($p < 0.01$) in the diabetics (mean $218.5 \pm SE 25.3$ ng/l) than in the controls (127.7 ± 9.8 ng/l), but this increase was not related to microangiopathy. The ability of platelets to generate thromboxane B_2 did not differ between the diabetics (181.4 ± 16.4 µg/l) and controls (195.8 ± 11.8 µg/l). Platelets of diabetics with microangiopathy or taking oral hypoglycaemic agents ($n = 19$), however, produced decreased amounts of thromboxane B_2 during clotting. Plasma concentrations of 6-keto-PGF $_{1\alpha}$ and thromboxane B_2 were not related to concentrations of glucose, haemoglobin A_{1c} , high-density lipoprotein cholesterol, cholesterol, triglycerides, magnesium, or creatinine.

These results suggest that in diabetics with microangiopathy a balance between prostacyclin and thromboxane A_2 is shifted to dominance by prostacyclin.

Introduction

The increased adhesiveness and aggregability of platelets in diabetes mellitus may be related to the development of diabetic angiopathy, but the causes of these platelet abnormalities are not known.¹⁻³ Diminished production of potent antiaggregatory prostacyclin by the vascular wall⁴ shown by some in-vitro studies in experimental^{5,6} and human diabetes^{7,8} has now been disputed.⁹ Other studies have shown an increased generation of proaggregatory thromboxane A_2 ¹⁰ by diabetic platelets in vitro.¹¹⁻¹³ Because the balance between prostacyclin and thromboxane A_2 rather than the absolute concentrations of these substances may be crucial in the interactions between platelets and blood-vessel walls,¹⁴ we measured circulating concentrations of the stable hydration products of prostacyclin and thromboxane A_2 —namely, 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PGF $_{1\alpha}$) and thromboxane B_2 ^{10,15}—in plasma from 53 diabetics and 53 controls matched for age and sex. We also studied the ability of platelets to produce thromboxane B_2 during spontaneous clotting of the blood.

Subjects and methods

The 53 diabetics (21 men, 32 women) were aged 18-76 years (mean $53.1 \pm SE 3.1$ years) (table I). Fourteen had juvenile-onset diabetes and 39 adult-onset disease, and the duration of diabetes varied from two months to 33 years (mean $14.7 \pm SE 1.3$ years). Thirty-four were receiving once- or twice-daily subcutaneous insulin with a mean dose of 45.9 ± 2.2 units/70 kg, and 19 were being treated with oral hypoglycaemic agents (glipizide in 12, metformin hydrochloride in four, and glibenclamide in three). No ketonuria was detected in any patient. Twenty-eight patients had diabetic microangiopathy, defined as

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proliferative retinopathy (six patients) or background retinopathy (11) with or without albuminuria (12). Fifty-three subjects with no evidence of glucose intolerance were matched for age and sex and served as controls (table I). No subject had taken drugs known to interfere with prostaglandin synthesis or metabolism in the previous 10 days, but 29 diabetics and 16 controls were taking various anti-hypertensive drugs (dihydrallazine, methyldopa, beta-blockers, or diuretics) or digoxin.

After an overnight fast blood samples (10 ml) were collected by venepuncture with minimal venous occlusion into ice-cold heparinised tubes containing indomethacin at a final concentration of 10 µmol/l. The plasma was separated immediately by centrifugation at 4°C and frozen until assayed. Thromboxane B₂ was measured using an established radioimmunoassay.¹⁶ For measurement of 6-keto-PGF_{1α} concentration plasma was acidified to pH 3 with hydrochloric acid and extracted four times with ethyl acetate. The combined

Blood specimens were also taken from 20 of the diabetics and haemoglobin A₁ (HbA_{1c}) concentration measured spectrophotometrically after haemolysation of the sample and separation of the glycosylated haemoglobins with ion-exchange chromatography. Concentrations of glucose, serum triglycerides, cholesterol, high-density-lipoprotein cholesterol (HDL cholesterol), magnesium, and creatinine were also measured using routine laboratory methods.

The Mann-Whitney rank sum test and regression analysis were used for statistical analysis of the results.

Results

Plasma concentrations of both 6-keto-PGF_{1α} and thromboxane B₂ were higher in the diabetics than the controls (table I). Plasma concentrations of 6-keto-PGF_{1α} were higher in the diabetics with micro-

TABLE I—Plasma concentrations of 6-keto-PGF_{1α} and thromboxane B₂ (mean ± SE) in diabetics and controls

	Mean age (range)	F:M	6-keto-PGF _{1α} (ng/l)	Signifi- cance	Thromboxane B ₂ (ng/l)	Signifi- cance
All diabetics (n = 53)	53.1 (18–76)	32:21	107.7 ± 7.6*		218.5 ± 25.3**	
Angiopathy (n = 28)	47.0 (21–73)	17:11	124.3 ± 10.8***	} p < 0.01	246.3 ± 42.3***	} NS
No angiopathy (n = 25)	58.2 (18–76)	15:10	89.2 ± 9.3		187.9 ± 25.4*	
Insulin treatment (n = 34)	46.1 (18–75)	20:14	126.1 ± 10.5***	} p < 0.001	223.8 ± 32.4**	} NS
Oral hypoglycaemic agents (n = 19)	64.7 (49–76)	12:7	74.9 ± 2.1		210.2 ± 41.7*	
Juvenile-onset disease (n = 14)	28.6 (18–56)	10:4	135.0 ± 17.2**	} p < 0.01	216.1 ± 40.2*	} NS
Adult-onset disease (n = 39)	61.3 (21–76)	22:17	97.6 ± 11.4		223.4 ± 34.2**	
Controls (n = 53)	52.2 (18–76)	32:21	87.5 ± 4.7		127.7 ± 9.8	

Patients versus controls: *p < 0.05; **p < 0.01; ***p < 0.001.

TABLE II—Release of thromboxane B₂ during spontaneous clotting of blood and ratio of plasma 6-keto-PGF_{1α} to serum thromboxane B₂ (× 10³) in same subjects

	Mean age (range)	F:M	Thromboxane B ₂ (µg/l)	Signifi- cance	6-keto-PGF _{1α} : thromboxane B ₂ (× 10 ³)	Signifi- cance
Diabetics (n = 33)	57.9 (18–76)	18:15	181.4 ± 16.4		0.47 ± 0.04	
Angiopathy (n = 10)	60.1 (29–71)	5:5	152.7 ± 35.9*	} NS	0.61 ± 0.09	} p < 0.05
No angiopathy (n = 23)	56.7 (18–76)	13:10	194.4 ± 17.9		0.41 ± 0.03	
Insulin treatment (n = 14)	49.1 (18–70)	6:8	221.5 ± 32.7	} NS	0.39 ± 0.06	} NS
Oral hypoglycaemic agents (n = 19)	64.7 (49–76)	12:7	153.9 ± 14.9*		0.52 ± 0.05	
Glipizide (n = 12)	66.9 (55–76)	9:3	146.0 ± 12.7		0.59 ± 0.06	
Metformin hydrochloride (n = 4)	62.5 (58–71)	2:2	169.8 ± 64.5		0.37 ± 0.11	
Glibenclamide (n = 3)	62.3 (49–74)	1:2	164.0 ± 11.8		0.41 ± 0.04	
Controls (n = 33)	58.6 (19–75)	18:15	195.8 ± 11.8		0.45 ± 0.03	

*Patients versus controls: p < 0.05.

organic phases were evaporated to dryness under a nitrogen stream. Two different parts (both in duplicate) of the dry residue dissolved in methanol were taken for saturation analysis, using specific antibodies raised in rabbits against a conjugate of 6-keto-PGF_{1α} and bovine serum albumin and a tritiated tracer (New England Nuclear Corporation, Boston, USA). The specificity of the antibody was tested against 22 other prostaglandins or related compounds. All cross-reactions were less than 0.1% at the 50% displacement level, except for that with 6-keto-prostaglandin E₁, which was 1.8%. The recovery of 6-keto-PGF_{1α} (50–200 ng/l) added to plasma was 84–104% (n = 15), and the coefficients of intra-assay and interassay variations were 6.8–8.5% and 9.3–14.2%, respectively. Measurement of plasma 6-keto-PGF_{1α} concentration as an index of prostacyclin production was further validated by showing a linear relation (r = 0.958, p < 0.001, n = 24) between doses (1–8 ng/kg/min) of prostacyclin infused into six healthy adults and the rises in plasma 6-keto-PGF_{1α} concentrations. We also showed in vitro that release of radioimmunoassayable 6-keto-PGF_{1α} by umbilical cord vessels was inhibited by indomethacin in a concentration-dependent manner.

The ability of platelets to produce thromboxane B₂ during spontaneous clotting was studied in 33 consecutive diabetics (cases 21–53). A blood sample (3 ml) was taken into a dry test tube and allowed to clot at 37°C for 60 minutes. The serum concentration of thromboxane B₂ was measured as an indicator of the ability of the platelets to generate thromboxane A₂. The amount of thromboxane B₂ produced during spontaneous clotting correlated closely (r > 0.9) with the amount of thromboxane B₂ released from platelets during induced aggregation in platelet-rich plasma.¹⁶

angiopathy, those receiving insulin, and those with juvenile-onset disease. The ability of platelets to generate thromboxane B₂ during clotting was decreased in the diabetics with microangiopathy and in those taking oral hypoglycaemic drugs (table II). The ratio of plasma 6-keto-PGF_{1α} to serum thromboxane B₂ was similar in diabetics and controls but was higher in patients with angiopathy than in those without angiopathy (table II).

Plasma concentrations of 6-keto-PGF_{1α} and thromboxane B₂ were not related to the age of the diabetics, the dose of insulin, or the use of other drugs; to the concentrations of glucose, HbA_{1c}, cholesterol, HDL cholesterol, triglycerides, magnesium, and creatinine; or to creatinine clearance.

Discussion

We studied the generation of prostacyclin and thromboxane A₂ in diabetics with and without vascular complications by measuring the circulating concentrations of 6-keto-PGF_{1α} and thromboxane B₂ and the ability of platelets to produce thromboxane B₂ during spontaneous clotting of the blood. During the study the volunteers continued to take insulin, oral hypoglycaemic agents, and drugs for cardiovascular diseases because to stop these drugs for research purposes would have been unethical. Since no data exist showing that these drugs might interfere with the synthesis or metabolism of prostacyclin or

thromboxane A₂ we believe that the diabetes itself or its complications caused the changes seen in this study.

Our data, together with the report of Davis *et al.*,⁹ challenge the theory that diminished synthesis of prostacyclin is related to diabetic angiopathy as suggested by in-vitro⁵⁻⁸ and in-vivo¹⁷ studies. We found that production of prostacyclin was increased in severe angiopathy, but in view of the effects of prostacyclin^{4 14} this rise per se is unlikely to cause the angiopathy. Platelets are more active in severe than mild diabetes,¹⁻³ so we assume that the increase in prostacyclin and the activation of platelets are connected.

The mechanism of the increased production of prostacyclin in diabetic angiopathy is hard to deduce. The activated platelets might release more endoperoxides to the vascular wall, which, in turn, might increase the production of prostacyclin to prevent thrombosis.^{14 18} Alternatively, some other secondary factor in the hyperactivity of the platelets might increase the synthesis of prostacyclin. Such a factor could be, for example, thrombin, which is released locally by microthrombi and may stimulate synthesis of prostacyclin.¹⁹ The biological importance of the rise in prostacyclin is likely to be a vascular "self-protection" against platelet adhesion and aggregation. The stimulated output of prostacyclin in diabetic angiopathy may be analogous to the increased generation of prostacyclin in spontaneously hypertensive rats²⁰ or during cardiopulmonary bypass in man.²¹

The increased circulating concentrations of thromboxane B₂ seen in the diabetics in our study are compatible with platelet activation¹⁻³ or with increased synthesis of thromboxane A₂ by diabetic platelets in vitro,¹¹⁻¹³ but the normal or even decreased production of thromboxane B₂ in patients with diabetic angiopathy during spontaneous clotting was unexpected. We speculate that the diabetic platelets have emptied their stores of endoperoxides and other precursors of prostaglandin synthesis—for example, for the increased circulating thromboxane B₂ or vascular synthesis of prostacyclin, or both. This may result in decreased release of thromboxane B₂ in serum when synthesis of thromboxane A₂ takes place in the absence of exogenous substrate. This finding does not disagree with increased biosynthesis of thromboxane A₂ by diabetic platelets from exogenous arachidonic acid in vitro.¹¹⁻¹³

We found no relations between plasma concentrations of 6-keto-PGF_{1α} and thromboxane B₂ and concentrations of glucose, HbA_{1c}, serum lipids, or magnesium, though diabetic angiopathy has been connected with the poor control of diabetes,²² changes in serum lipid concentrations,²³ and hypomagnesaemia.²⁴

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As the articulation of the ribs with the back-bone is very strong, they are not often dislocated. It does however sometimes happen, which is a sufficient reason for our taking notice of it. When a rib is dislocated either upwards or downwards, in order to replace it, the patient should be laid upon his belly on a table, and the operator must endeavour to push the head of the bone into its proper place. Should this method not succeed, the arm of the disordered side may be suspended over a gate or ladder, and, while the ribs are thus stretched asunder, the heads of such as are out of place may be thrust into their former situation.

THOSE dislocations wherein the heads of the ribs are forced inwards, are both more dangerous and the most difficult to reduce, as neither the hand nor any instrument can be applied internally to direct the luxated heads of the ribs. Almost the only thing that can be done is, to lay the patient upon his belly over a cask, or some gibbous body, and to move the fore-part of the rib inward towards the back, sometimes flaking it; by this means the heads of the luxated ribs may flip into their former place.

THE humerus or upper bone of the arm may be dislocated in various directions: It happens however most frequently downwards, but very seldom directly upwards. From the nature of its articulation, as well as from its exposure to external injuries, this bone is the most subject to dislocation of any in the body. A dislocation of the humerus may be known by a depression or cavity on the top of the shoulder, and an inability to move the arm: When the dislocation is downward or forward, the arm is elongated, and a ball or lump is perceived under the arm-pit; but when it is backward, there appears a protuberance behind the shoulder, and the arm is thrown forwards toward the breast.

(Buchan's *Domestic Medicine*, 1786.)