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Suppression of the Accumulation of Triosephosphates and Increased Formation of Methylglyoxal in Human Red Blood Cells during Hyperglycaemia by Thiamine *In Vitro*

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The accumulation of triosephosphates and the increased formation of the potent glycating agent methylglyoxal in intracellular hyperglycaemia are implicated in the development of diabetic complications. A strategy to counter this is to stimulate the anaerobic pentosephosphate pathway of glycolysis by maximizing transketolase activity by thiamine supplementation, with the consequent consumption of glyceraldehyde-3-phosphate and increased formation of ribose-5-phosphate. To assess the effect of thiamine supplementation on the accumulation of triosephosphates and methylglyoxal formation in cellular hyperglycaemia, we incubated human red blood cell suspensions (50% v/v) in short-term culture with 5 mM glucose and 50 mM glucose in Krebs-Ringer phosphate buffer at 37°C as models of cellular metabolism under normoglycaemic and hyperglycaemic conditions. In hyperglycaemia, there is a characteristic increase in the concentration of the triosephosphate pool of glycolytic intermediates and a consequent increase in the concentration and metabolic flux of the formation of methylglyoxal. The addition of thiamine (50–500 μM) increased the activity of transketolase, decreased the concentration of the triosephosphate pool, decreased the concentration and metabolic flux of the formation of methylglyoxal, and increased the concentration of total sedoheptulose-7-phosphate and ribose-5-phosphate. Biochemical changes implicated in the development of diabetic complications were thereby prevented. This provides a biochemical basis for high dose thiamine therapy for the prevention of diabetic complications.

Key words: diabetic complications, hyperglycaemia, methylglyoxal, thiamine, triosephosphates.

In recent clinical trials investigating the effects of conventional and intensive therapy for hyperglycaemia associated with Type 1 and Type 2 diabetes mellitus, hyperglycaemia is an independent risk factor for the development of microvascular complications (*1, 2*). The biochemical basis of the link between hyperglycaemia and the development of diabetic complications remains uncertain, but is the subject of several hypotheses currently under experimental examination: namely, those implicating a role for the activation of protein kinase C_β (*3*), "metabolic pseudohypoxia" (*4*), the activation of the polyol pathway (*5*), oxidative stress (*6*), and the accumulation of advanced glycation endproducts (AGEs) (*7, 8*). The dysfunctional physiological states described in these hypotheses occur as a direct or indirect consequence of the abnormal metabolism of glucose arising from episodes of hyperglycaemia. The importance of intracellular hyperglycaemia in these events has been demonstrated by experiments in which the overexpression of the GLUT1 glucose transporter in vascular pericytes produces cytosolic hyperglycaemia and changes in cell function characteristic of the diabetic phenotype (*9*). The accumulation of triosephosphates in pericytes and endothelial cells is a critical feature of the metabolic dysfunction and consequences of intracellular hyperglycaemia (*10*). This accumulation leads to the increased formation of glycerol-3-phosphate (G-3-P), diacylglycerol and the activation of protein kinase C_β (*3*), and to an increased formation of methylglyoxal (*11*), a reactive α-oxoaldehyde metabolite important in the cellular and extracellular formation of AGEs (*12*). Triosephosphate accumulation may be caused by the *in situ* inhibition of glyceraldehyde-3-phosphate dehydrogenase (GA3PDH) in metabolic pseudohypoxia, which may develop from flux through the polyol pathway (*4*), and as a consequence of the oxidative inactivation of GA3PDH (*13*) (Fig. 1). If triosephosphate accumulation in hyperglycaemia could be prevented, it might offer a new preventive therapy against diabetic complications.

A strategy to achieve this is to activate the non-oxidative pentosephosphate pathway in which glyceraldehyde-3-

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Abbreviations: AGE, advanced glycation endproduct; 1,3-bis-PG, 1,3-bisphosphoglycerate; 3-DG, 3-deoxyglucosone; DHAP, dihydroxyacetonephosphate; F-1,6-bis-P, fructose-1,6-bis-phosphate; F-6-P, fructose-6-phosphate; G-6-P, glucose-6-phosphate; G3P, glycerol-3-phosphate; GA3P, glyceraldehyde-3-phosphate; GA3PDH, glyceraldehyde-3-phosphate dehydrogenase; PEP, phosphoenolpyruvate; 2-PG, 2-phosphoglycerate; 3-PG, 3-phosphoglycerate; PKC_β, protein kinase C_β; RBC, red blood cell; R-5-P, ribose-5-phosphate; TPI, triosephosphate isomerase; TPP, thiaminepyrophosphate.

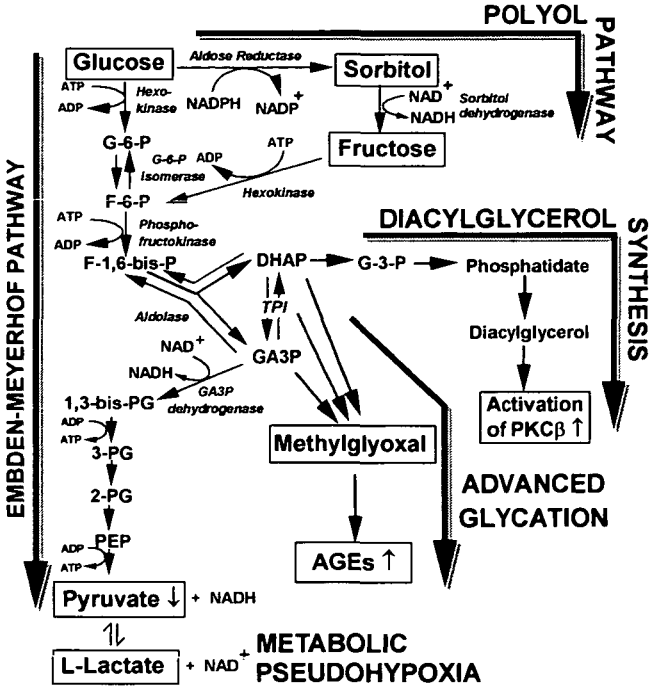

Fig. 1. **Biochemical changes in Embden-Meyerhof glycolysis during hyperglycaemia associated with vascular dysfunction in diabetes mellitus.**

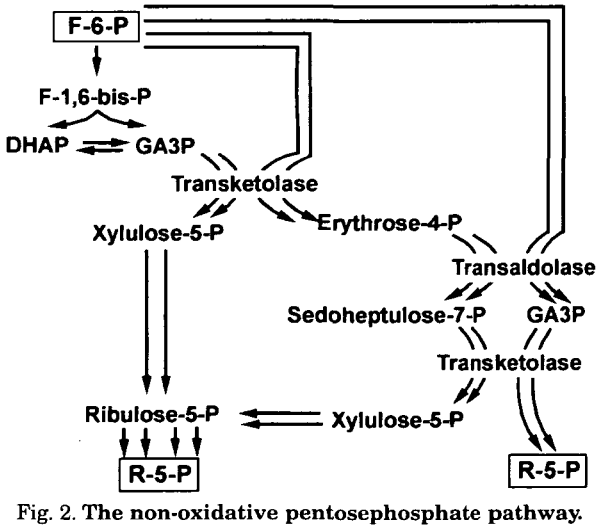

Fig. 2. **The non-oxidative pentosephosphate pathway.**

phosphate (GA3P) and fructose-6-phosphate (F-6-P) are converted to ribose-5-phosphate (R-5-P): 2 GA3P + 4 F-6-P → 6 R-5-P (Fig. 2). This can be achieved by high dose thiamine supplementation, which maximizes the activity of transketolase with a saturating concentration of thiamine-pyrophosphate (TPP) cofactor. Increased R-5-P may stimulate *de novo* purine synthesis by increasing the availability of ribosylpyrophosphate. R-5-P is a glycating agent, but is much less reactive than methylglyoxal and is present at low concentration (20 μM) in cells. Thiamine supplementation should produce a decrease in triosephosphate pool metabolites and a concomitant increase in R-5-P. In this report, we describe the first demonstration of this effect.

TABLE I. **Concentrations of L-lactate and pyruvate in 50% human red blood cell suspensions under normoglycaemic and hyperglycaemic conditions *in vitro*.**

Incubation			[L-Lactate]	[Pyruvate]	
[Glucose]$_o$ (mM)	[Thiamine]$_o$ (μM)	n	Mean ± SD (μM)	Mean ± SD (μM)	[Lactate]/[Pyruvate]
5 (t = 0)	0	6	512 ± 67	25 ± 10	—
5 (t = 2 h)	0	6	2935 ± 524	263 ± 38	11.2 ± 2.6
5 (t = 2 h)	500	3	2564 ± 370	236 ± 12	10.9 ± 1.7
50 (t = 2 h)	0	6	3164 ± 472	179 ± 16†	17.7 ± 3.1‡
50 (t = 2 h)	500	3	2818 ± 434	190 ± 9‡	14.8 ± 2.4

Significance: †$p < 0.01$ and ‡$p < 0.05$ with respect to normoglycaemic control. Red blood cells (50% v/v) were incubated in Krebs-Ringer phosphate buffer, pH 7.4, at 37°C for 2 h with the concentrations of glucose and thiamine indicated.

MATERIALS AND METHODS

Materials—Thiamine, TPP, erythrose-4-phosphate, R-5-P, NAD+, NADH, fructose-1,6-bisphosphate (F-1,6-bis-P), D-lactic dehydrogenase [EC 1.1.1.28] from *Staphylococcus epidermidis*, L-lactic dehydrogenase [EC 1.1.1.27], GA3PDH [EC 1.2.1.12], α-glycerophosphate dehydrogenase [EC 1.1.1.8; GDH], and aldolase [EC 4.1.2.13] from rabbit muscle, triosephosphate isomerase [EC 5.3.1.1; TPI], ribulose-5-phosphate 3-epimerase [EC 5.1.3.1], and transketolase [EC 2.2.1.1] from Bakers yeast, and phosphoriboisomerase [EC 5.3.1.6] from spinach were purchased from Sigma.

Isolation of Human Red Blood Cells—Human RBCs were isolated from blood samples (50 ml) drawn with informed consent of the subject by venous puncture into tubes containing ethylenediaminetetra-acetic acid anticoagulant. Blood samples were centrifuged (2,000 ×*g*, 5 min) and the plasma and white blood cells were removed. The packed RBC pellet was washed three times with 4 volumes of phosphate-buffered saline (1 part 100 mM NaH_2PO_4/Na_2HPO_4, pH 7.4; 9 parts 0.9% NaCl), and washed a fourth and final time with 4 volumes of Krebs-Ringer phosphate buffer (120 mM NaCl, 4.8 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgSO_4$, 16.5 mM NaH_2PO_4/Na_2HPO_4, pH 7.4, and 5 mM β-D-glucose).

RBCs (50%, v/v) were incubated in Krebs-Ringer phosphate buffer with 5 mM glucose (normoglycaemic model) or 50 mM glucose (hyperglycaemic model), with or without 50–500 μM thiamine for 2 h at 37°C. In samples including 50 mM glucose, isotonicity was preserved by decreasing the concentration of NaCl. After incubation, the cell suspensions were processed for metabolite analysis and assay of transketolase activity. For the D- and L-lactate, pyruvate, triosephosphate, and pentosephosphate pathway metabolite assays, perchloric acid extracts were prepared by adding 1 ml of ice-cold 0.6 M perchloric acid to the cell suspensions; the samples were left on ice for 10 min and the precipitates were then sedimented by centrifugation (6,000 ×*g*, 10 min, 4°C). For assay of the concentrations of α-oxoaldehydes methylglyoxal and 3-deoxyglucosone (3-DG), a similar perchloric acid extract was prepared, except that prior to centrifugation, an internal standard 6,7-dimethoxy-2-ethyl-3-methylquinoxaline (5 μM in 0.5 M HCl; 20 μl) was added. Zero time samples were 50% red blood cell incubations inactivated with ice-cold perchloric acid before the addition of glucose. For the assay of transketolase activity,

a lysate was prepared: RBCs were sedimented by centrifugation and the supernatant removed; the RBCs were lysed with 4 volumes of water and the membrane fragments sedimented (10,000 ×*g*, 10 min, 4°C).

Metabolite Assays—The concentrations of methylglyoxal and 3-DG were assayed in RBCs and extracellular medium by derivatization with 1,2-diamino-4,5-dimethoxybenzene and HPLC of the quinoxaline adducts (*14*). The concentrations of D- and L-lactate in red blood cell suspensions were determined by the endpoint enzymatic assay procedure as described (*15*), adapted for microplate-based assay, with absorbance detection at 340 nm using D-lactic dehydrogenase and L-lactic dehydrogenase, respectively. The concentration of pyruvate was assayed similarly by the method of Beutler (*16*). The concentrations of the triosephosphate pool of metabolites (total [GA3P] + [DHAP] + 2 × [F-1,6-bis-P]) were determined, in the presence of sodium arsenate, by endpoint enzymatic assay with GA3PDH, TPI and aldolase, respectively, by fluorimetric detection of NADH (*17*). Total triosephosphate concentration is a convenient measure of triosephosphate accumulation since GA3P, DHAP, and F-1,6-bis-P are considered to be in dynamic equilibrium, although evidence has been found for some metabolic channelling of GA3P to GA3PDH in the Embden-Meyerhof pathway (*18*).

The concentrations of pentosephosphate pathway metabolites, xylulose-5-phosphate, ribulose-5-phosphate, and combined R-5-P + sedoheptulose-7-phosphate, were determined by endpoint enzymatic assay by a modification of the method of Casazza and Veech (*19*). RBC suspensions (1 ml) were deproteinised by the addition of ice-cold perchloric acid (1 ml, 0.6 M), and the extracts were left on ice for 10 min and then centrifuged (6,000 ×*g*, 10 min, 4°C). Aliquots of the supernatants (1 ml) were removed and neutralized to pH 7 with 1 M K_2CO_3. Aliquots of neutralized extracts (140 μl) were added to assay cocktail (30 mM NAD+, 18 mM $MgCl_2$, 15 mM sodium arsenate, 250 μM erythrose-4-phosphate, 719 μM TPP, and 152 mM imidazole, pH 7.6; 70 μl) and GA3PDH (140 U/ml; 10 μl) in the wells of a 96-well microplate. The formation of NADH was quantified by measuring the absorbance at 340 nm and interpolating on a calibration curve of 0–300 μM NADH. Transketolase (5 U/ml; 10 μl), ribulose-5-phosphate-3-epimerase (25 U/ml; 10 μl), and phosphoriboisomerase (50 U/ml; 10 μl) were added sequentially, with the final steady absorbance monitored after each addition achieved after intervals of 10, 5, and 45 min, respectively. Blanks contained either no cell extract or no enzymes. The increases in NADH concentration determined where used to deduce the concentrations of xylulose-5-phosphate, ribulose-5-phosphate, and combined R-5-P + sedoheptulose-7-phosphate combined.

Assay of Transketolase Activity—The activity of transketolase in RBCs was determined by the method of Chamberlain *et al.* (*20*). Aliquot (200 μl) of substrate cocktail (14.8 mM R-5-P, 253 μM NADH, 185 U/ml TPI, and 70 μl of 21.5 U/ml GDH in 250 mM Tris/HCl buffer, pH 7.8) were added to the wells of a 96-well microplate and 20 μl of a 6-fold dilution of RBC lysate was added. The absorbance at 340 nm was monitored at 10 min intervals for 2 h and the rate of decrease in absorbance between 20 to 80 min was used to deduce the rate of oxidation of NADH in the GDH catalyzed reaction, which is rate limited by the transketolase catalyzed conversion of R-5-P and xylulose-5-phosphate to sedoheptulose-7-phosphate and GA3P under these conditions. The maximum rate of transketolase under TPP saturation conditions was determined by adding 350 μM TPP to the assay incubation mixture.

Statistical Analysis of Results—Significance of changes in measured variables was assessed by Student's *t* test.

RESULTS

Effect of Model Hyperglycaemia on the Concentration of Triosephosphate Pool Glycolytic Intermediates and Methylglyoxal Formation in Human Red Blood Cells In Vitro—When 50% (v/v) RBC suspensions were incubated for 2 h with 50 mM glucose under model hyperglycaemic conditions, the concentration of the triosephosphate pool of glycolytic intermediates increased *ca.* 2-fold relative to incubations with 5 mM glucose, the normoglycaemic control ($p < 0.001$)—Fig. 3a. The addition of 500 μM thiamine to incubations with 50 mM glucose, however, resulted in a significant decrease in the concentration of the triosephosphate pool glycolytic intermediates ($p < 0.001$). The hyperglycaemic model was associated with a significant decrease in pyruvate concentration ($p < 0.001$), no significant change in L-lactate concentration, but a significant increase in the [L-lactate]/[pyruvate] ratio ($p < 0.05$). The addition of thiamine did not prevent the decrease in pyruvate concentration in the hyperglycaemic model, but the L-lactate concentration was also decreased and, therefore, the [L-lactate]/[pyruvate] ratio did change significantly with respect to the normoglycaemic control value with or without thiamine ($p > 0.05$).

A consequence of the increased concentration of triosephosphates in hyperglycaemia was the increased formation of methylglyoxal, formed mainly by the non-enzymatic degradation of triosephosphates (*21*). The concentration of methylglyoxal increased in RBC suspensions incubated with 50 mM glucose, relative to the 5 mM normoglycaemic control ($p < 0.01$). This increase was prevented in the presence of 500 μM thiamine—Fig. 3b. Methylglyoxal may react directly with thiamine to decrease methylglyoxal concentration in hyperglycaemic model cultures. The rate of reaction of methylglyoxal (500 nM) with 50–500 μM thiamine in Krebs-Ringer phosphate buffer, pH 7.4, at 37°C was investigated, and the rate constant was found to be 2.36 ± 0.33 $M^{-1} min^{-1}$ ($n = 12$).

The fate of >99% of methylglyoxal formed in RBCs is metabolism by the glyoxalase system to D-lactate (*22*), which is not metabolized further in RBCs (*23*). The flux of methylglyoxal formation may be estimated by measuring the increase in D-lactate concentration in RBC suspensions (*24*). The D-lactate concentration increased significantly during incubation with 5 mM glucose ($p < 0.001$), the normoglycaemic model, reflecting the flux in the formation of methylglyoxal. In hyperglycaemic models of RBCs incubated with 20 and 50 mM glucose, the concentration of D-lactate increased *ca.* 1-fold more than with 5 mM glucose ($p < 0.001$)—Fig 3c. An osmotic control experiment in which RBCs were incubated with 5 mM glucose and 45 mM mannitol resulted in an increase in D-lactate concentration of 1.9 ± 1.6 μM (14%) above the normoglycaemic control value, a result that was not significant ($p > 0.05$). The addition of 500 μM thiamine to both hyperglycaemic cultures decreased the concentration of D-lactate to that in the normoglycaemic control ($p < 0.001$). The dependence of this

effect on the concentration of thiamine was investigated. Thiamine caused a decrease in the formation of D-lactate in the concentration range 50–500 μM—Fig. 3d.

The effect of hyperglycaemia on the accumulation of 3-DG was also investigated. The concentration of 3-DG in red cell suspensions increased in hyperglycaemic culture, and this increase was not prevented in the presence of 500 μM thiamine—Fig. 4a.

Thiamine enters RBCs by facilitated diffusion and is phosphorylated to thiamine monophosphate and TPP in *ca.* 30 min under physiological conditions (*25*). The effect of thiamine supplementation on the transketolase activity of RBCs in culture was investigated (Fig. 4b, incubations N+T and H+T *versus* incubations N and H, respectively), in comparsion with the limiting, TPP-saturating value of transketolase, which was determined by adding of 250 μM TPP to RBC lysates from normoglycaemic and hyperglycaemic cultures (Fig. 4b, N+TPP and H+TPP, respectively). Incubation of RBCs with 500 μM thiamine resulted in an increase in the activity of transketolase to the maximum value found in the presence of TPP. This occurred in both normoglycaemic and hyperglycaemic incubations. The effect of increased transketolase activity on the concentrations of metabolites in the pentosephosphate pathway was also investigated. Pentosephosphate pathway metabolites were conveniently determined by endpoint enzymatic assay. This enabled the measurement of the concentrations of xylulose-5-phosphate, ribulose-5-phosphate, and combined R-5-P and sedoheptulose-7-phosphate (*19*). Thiamine supplementation increased the combined R-5-P and sedoheptulose-7-

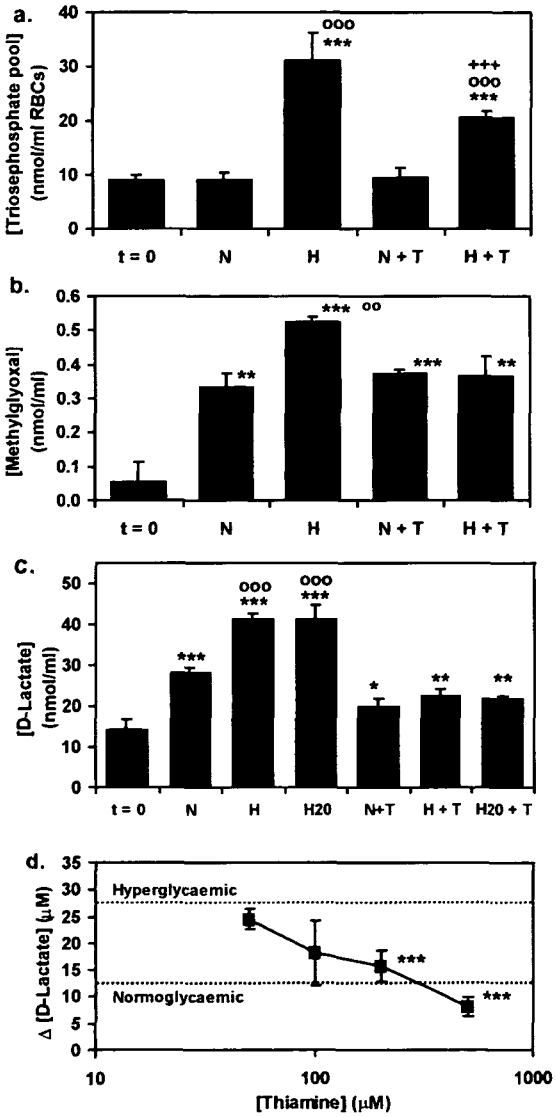

Fig. 3. **Effect of thiamine on the concentration of (a) total triosephosphates, (b) methylglyoxal, and (c) D-lactate in human red blood cell suspensions *in vitro*. Dose response effect of thiamine on D-lactate formation (d).** Key: t = 0, zero time estimate; N, + 5 mM glucose; H, + 50 mM glucose; H20, + 20 mM glucose; N + T, + 5 mM glucose and 500 μM thiamine; H + T, + 50 mM glucose and 500 μM thiamine; and H20 + T, + 20 mM glucose and 500 μM thiamine. Significance: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, with respect to t = 0; $^{oo}p < 0.01$ and $^{ooo}p < 0.001$ with respect to N; $^{+++}p < 0.001$ with respect to H. Data are mean ± SD of 3–9 determinations.

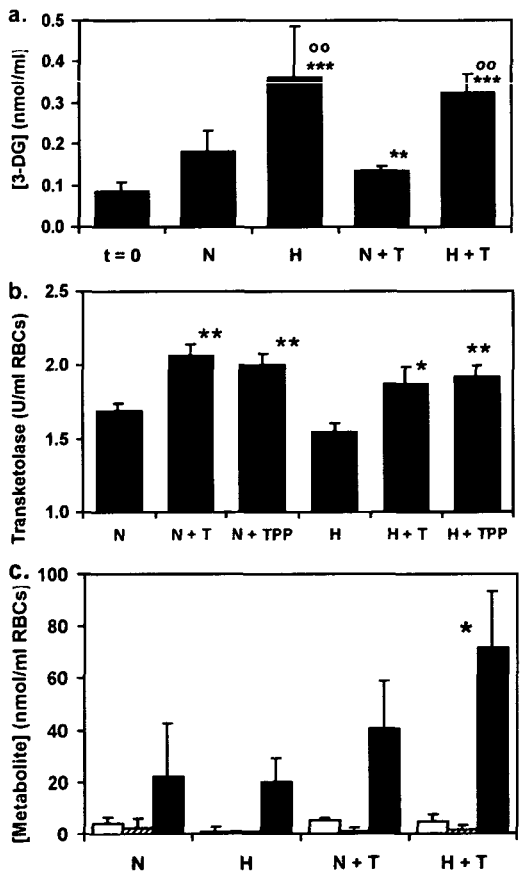

Fig. 4. **Effect of thiamine on (a) 3-deoxyglucosone concentration, (b) transketolase activity, and (c) pentosephosphate metabolites in human red blood cell suspensions *in vitro*.** Key: N, + 5 mM glucose; H, + 50 mM glucose; N + T, + 5 mM glucose and 500 μM thiamine; and H + T, + 50 mM glucose and 500 μM thiamine; and a. N + TPP, + 5 mM glucose incubation with 350 μM TPP added to diluted lysate; H + TPP, + 50 mM glucose incubation with 350 μM TPP added to diluted lysate; and b. bar filling key: □ xylulose-5-phosphate, ▨ ribulose-5-phosphate, and ■ combined sedoheptulose-7-phosphate and R-5-P. Significance: a. $^{**}p < 0.01$ and $^{***}p < 0.001$, with respect to t = 0; $^{oo}p < 0.01$ with respect to N. b. and c. $^{*}p < 0.05$, $^{**}p < 0.01$, with respect to non-thiamine supplemented control. N. Data are mean ± SD of 3–6 determinations.

phosphate concentration significantly in hyperglycaemic incubations ($p < 0.05$) —Fig. 4c.

DISCUSSION

When RBCs are incubated under hyperglycaemic conditions in short-term culture, the rate of glucose consumption increases and the flux of metabolites through the pentosephosphate pathway increases *ca.* onefold (*24, 26*). There is a small decrease in the concentration of reduced glutathione (4%) that is not significant (*24*), which suggests that there is no significant cellular oxidative stress in short-term hyperglycaemic culture, although such stress may exist in diabetic subjects *in vivo* (*27, 28*). The concentration of the triosephosphate pool of glycolytic intermediates increases in hyperglycaemic culture, with the concentration of pyruvate decreasing and the ratio of [L-lactate]/[pyruvate] ratio increasing (*26*), a finding confirmed in this study. This is the state of metabolic pseudohypoxia as defined by Williamson (*4*). The triosephosphate pool of glycolytic intermediates in RBCs is increased *ca.* 2-fold in clinical diabetic subjects, with the increase attributed to the accumulation of F-1,6-bis-P ($p < 0.001$), and the [L-lactate]/[pyruvate] ratio is also increased ($p < 0.01$) (*29*). The accumulation of triosephosphates may be due to both increased metabolism of glucose to L-lactate and the transfer of reducing equivalents from the pentosephosphate pathway to the Embden-Meyerhof pathway by polyol pathway activity. Although the concentration of pyruvate was significantly decreased in the hyperglycaemic model including thiamine, the concentration of L-lactate was also decreased so that the [L-lactate]/[pyruvate] ratio was not significantly different from that of the normoglycaemic control value. Metabolic pseudohypoxia was lifted by the addition of thiamine in these studies, as defined by the [L-lactate]/[pyruvate] ratio. An alternative pathway for the metabolism of triosephosphates was activated that did not produce NADH and L-lactate.

The addition of thiamine to RBC suspensions suppressed the accumulation of triosephosphates in hyperglycaemia and reversed the accumulation and increased flux of the formation of methylglyoxal (Fig. 3, a–c). This effect of thiamine was found in model cultures of moderate and severe hyperglycaemia (20 and 50 mM glucose, respectively) suggesting that this effect may be relevant to the diabetic state *in vivo*. Similar effects were expected since glucose transport into RBCs via the GLUT1 glucose transporter is saturated in moderate and severe hyperglycaemia (*30*). The addition of 45 mM mannitol to normoglycaemic cultures did not produce a similar effect, indicating that these changes in metabolites are linked to the metabolism of glucose in hyperglycaemia and not to hypertonic effects.

Thiamine reacted irreversibly with methylglyoxal but the reaction kinetics were so slow that the predicted rate of scavenging of methylglyoxal by 500 μM thiamine in RBC suspensions is only 0.6 nM min^{-1}. This only accounts for <1% of the decrease in the flux of methylglyoxal by thiamine in the hyperglycaemic culture for 2 h (*ca.* 20 μM). It is likely, therefore, that the decrease in concentration and flux of methylglyoxal formation in the hyperglycaemic culture produced by thiamine is due to the decreased concentration of triosephosphate precursors, G3AP and DHAP (*21*). Indeed, the increase in the concentration of a related α-oxoaldehyde, 3-DG, in hyperglycaemic culture was not prevented by thiamine. 3-DG is formed from fructose-3-phosphate by glycation reactions (*14, 31*) and these processes are not expected to be suppressed by thiamine. The addition of thiamine produced a concomitant increase in the combined sedoheptulose-7-phosphate and R-5-P concentration, however. This is consistent with a decrease in the triosephosphate pool mediated by the consumption of G3AP by the pentosephosphate pathway, driven by the maximization of transketolase activity.

Thiamine also decreased the rate of formation of D-lactate in normoglycaemic cultures (Fig. 3c). This effect may be due to hydrolysis of some thiamine to the corresponding mercapto-olefin derivative, reversible binding of methylglyoxal to this with a consequent decrease in the rate of detoxification of methylglyoxal by the RBC glyoxalase system.

Under normal conditions there is, in fact, a net generation of triosephosphates in the pentosephosphate pathway of RBCs, accounting for only *ca.* 1% of triosephosphate formation. When the pentosephosphate pathway is stimulated *ca.* 5-fold by an oxidant, the formation of triosephosphates by the pentosephosphate pathway also increases *ca.* 5-fold but still only accounts for *ca.* 8% of the total triosephosphate formation in red blood cells; total triosephosphate formation decreases by 11% during stimulation of the pentosephosphate pathway (*32*). This suggests that stimulation of the oxidative pentosephosphate pathway to produce NADPH does not lead to markedly decreased or increased changes in total triosephosphate concentrations. Increased utilization of NADPH by aldose reductase activity in the polyol pathway is, therefore, not sufficient to increase triosephosphate concentrations in hyperglycaemia. Herein, we have shown that stimulating transketolase activity to its maximum, TPP saturation-limited value leads to the activation of the non-oxidative pentosephosphate pathway where an increased proportion of the flux through the pentosephosphate pathway converts GA3P and F-6-P to R-5-P —Fig. 2. The beneficial effects of thiamine supplementation and the increase in transketolase activity in diabetes mellitus have been linked to an antioxidant activity brought about by the stimulation of the NADPH generation by the pentosephosphate pathway (*33*). This work suggests that the beneficial effects may rather be related to the net consumption of triosephosphates. The immediate consequences of the decrease in triosephosphate concentration caused by thiamine were the decreased formation and accumulation of methylglyoxal (*21, 24*) and the suppression of metabolic pseudohypoxia. There is an expectation that *de novo* diacylglycerol formation will be suppressed as well.

Supplementation of RBCs with thiamine has previously been shown to increase transketolase activity up to, but not beyond, the activity achieved with TPP saturation. This increase correlates with TPP concentration (*34, 35*). The most likely explanation for the increased transketolase activity in RBCs induced by thiamine is, therefore, an increased concentration of the TPP co-factor.

It is not known if similar effects of thiamine on glycolytic intermediates are produced to prevent triosephosphate accumulation in endothelial cells and pericytes of the retina, kidney and peripheral nerve microvessels, and peripheral neurones, in diabetes mellitus *in vivo*; nor that if these effects were found, they would suppress the development of diabetic complications. TPP is also a cofactor for pyruvate

dehydrogenase and α-ketoglutarate dehydrogenase, and increases in these enzymatic activities may also increase the disposal of glycolytic intermediates. Thiamine derivative therapy for diabetic complications is gaining increasing attention. Thiamine also prevents the hyperglycaemia-induced delayed replication of human umbilical and retinal endothelial cells *in vitro* (*36*). Streptozotocin-induced diabetic rats given 70 mg/kg thiamine or 100 mg/kg *S*-benzoylthiaminephosphate (Benfotiamine) daily for 6 months showed decreased development of diabetic neuropathy and advanced glycation (*37*). In recent clinical trials, Benfotiamine decreased the formation of methylglyoxal-derived AGEs (*38*) and suppressed the development of diabetic neuropathy (*39*). We also found effects of Benfotiamine on RBC metabolism in hyperglycaemia similar to those reported herein for thiamine (data not shown). Clinical diabetes mellitus is associated with a mild thiamine deficiency in some instances. In a study of diabetic subjects (type not specified), 76% had a thiamine concentration lower than the lower limit of the normal range (*40*). In a study of Type 2 diabetic subjects, 18% of subjects had a thiamine concentration lower than the lower limit of the normal range (*41*). When subjects were given 20–50 mg thiamine per day (20–50-fold the normal daily requirement), 14 of 24 subjects had the maximum, TPP-saturated transketolase activity (*40*). This work and related studies now provide a biochemical basis for the prevention of diabetic complications by high-dose thiamine therapy.

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