

Title of paper

High-dose thiamine supplementation improves glucose tolerance in hyperglycemic individuals:
a randomised, double – blind cross-over trial

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Abstract

Purpose To assess the effect of high-dose oral thiamine supplements on glucose tolerance in patients with impaired glucose metabolism.

Methods Twelve hyperglycemic subjects (10 cases of impaired glucose tolerance, 2 new cases of type 2 diabetes) completed this double-blind, randomised trial, where all participants received both placebo and thiamine capsules (3×100 mg/day) for six weeks in a cross-over manner. The main endpoint was changes in 2-h plasma glucose. Fasting plasma glucose and insulin, 2-h plasma insulin, the hemostatic model assessment of insulin resistance (HOMA-IR), renal function measurement and thiamin status were also evaluated at the commencement and completion of each treatment period.

Results Thiamine supplementation resulted in significant decreases in 2-h plasma glucose relative to baseline (8.78±2.20 mmol/l vs. 9.89±2.50, $p = 0.004$), with no significant change in the placebo arm. Fasting plasma glucose and insulin, and HOMA-IR increased significantly from baseline after 6 weeks in the placebo arm ($p = 0.003$, $p = 0.04$ and $p = 0.02$, respectively). These variables did not change with thiamine supplementation. There were no significant changes in 2-h plasma insulin or renal function marker, within or between arms.

Conclusion/interpretation Supplementation with high dose thiamine may improve glucose tolerance in patients with hyperglycemia. High dose thiamine supplementation may prevent or slow the progression of hyperglycemia toward diabetes mellitus in individuals with impaired glucose regulation.

Trial registration: Australian New Zealand Clinical Trials Registry ACTRN12611000051943

Key words Thiamine. Glucose tolerance. Insulin resistance. Hyperglycemia. Type 2 diabetes. Cardiovascular disease.

Abbreviations

eGFR	Estimated glomerular filtration rate
IGT	Impaired glucose tolerance
GFAT	Glutamine:fructose-6-phosphate amidotransferase
RBC	Red blood cell
TPP	Thiamine pyrophosphate
TRMA	Thiamine-responsive megaloblastic anemia

Introduction

Hyperglycemia is known to induce a variety of biochemical alterations at the cellular level, resulting in both vascular and tissue damage. Thiamine is a water soluble vitamin playing a key role as a co-enzyme in metabolic pathways involved in glucose metabolism. Existing evidence reveals that supplemental thiamine can reduce activity of certain biochemical pathways that lead to abnormalities associated with hyperglycemia [1]. Thiamine supplementation was reported to improve glucose tolerance in patients with hepatic cirrhosis [2]. Also, thiamine intake was shown to be inversely correlated to 2-h plasma glucose concentrations of subjects without diabetes [3]. Despite these preliminary findings, there have been no published studies to examine this relationship in individuals with impaired glucose tolerance or diabetes. Therefore, the main objective in this study was to assess the effect of high dose thiamine supplementation on glucose tolerance in patients with hyperglycemia at early stages. Additionally, the effects of supplemental thiamine on other metabolic measurements linked to hyperglycemia, including fasting plasma glucose and insulin, 2-h plasma insulin and HOMA-IR as well as renal function marker (GFR) and thiamine status were assessed at baseline and after each intervention period.

Material and methods

Subjects Seventeen hyperglycemic subjects (14 IGT, and 3 new cases of T2DM having 2-h plasma glucose levels ≥ 7.8 mmol/l) with a BMI 19-40 kg/m² and aged between 18-75 years commenced the study. Exclusion criteria were: smoking, known impaired renal or liver function, major gastrointestinal disorders, pregnancy or lactation and known allergy or intolerance to thiamine. No subject was on medications with possible effects on the study outcomes or thiamine metabolism. Subjects taking supplements containing thiamine or consuming more than 2 standard alcoholic drinks per day were asked to cease the supplement and reduce the alcohol intake during their participation, starting 4 weeks before attending the first clinical day.

Study design and procedure This was a randomised, double-blind, cross-over design trial. Hyperglycemic subjects were randomly allocated into two groups to consume either 100 mg thiamine (as thiamine hydrochloride) or placebo three times a day (300 mg/d) before meals for six weeks. Clinical measurements were made at baseline, week 3 and week 6. Following completion of the first part and a 14-week wash out period, subjects came back to receive alternative capsules for another six weeks. Subsequently, the measurements were repeated on another three separate visits according to the same protocol as the first part. All participants and investigators involved in data collection and analysis were blinded to the treatment assignments. The thiamine capsules contained thiamine hydrochloride as well as the inactive ingredients starch and lactose (Betamin, Sanofi-Aventis Australia Pty Ltd., Australia). The capsules provided as placebo were matched with the supplement and contained the same inactive ingredients.

Participants were asked to maintain their usual diet and level of physical activity during the study period. They were on an unrestricted carbohydrate diet of at least 150-200 g of carbohydrate/day for the three days before the glucose load, and consumed a standard meal provided by the investigators on the evening before the clinical days.

On these days, subjects attended the out-patient clinic, School of Public Health, Curtin University in the morning, after a 10-12 h overnight fast. Anthropometric measurements were taken with subjects dressed in a gown with no shoes and empty bladder. A 75-g oral glucose tolerance test was administered to all subjects at start and on completion of each arm part of the six week trial. Fasting and 2-h post glucose load blood samples were collected via venipuncture into serum and plasma tubes. Aliquots of plasma, serum and red blood cell (RBC) samples were stored at -80 °C until analysis.

This study was approved by the Curtin University Human Research Ethics Committee (Approval number HR 161/2008) and all participants provided written informed consent.

Statistical analysis All statistical analyses were performed using SPSS for Windows (version 16, SPSS Inc., Chicago). A paired sample t-test was used to compare the metabolic characteristics of subjects at the baseline in placebo and supplement arms. The effects of treatment (thiamine supplement) on cardiovascular risk factors were assessed using a linear mixed-model analysis, with treatment, treatment*week interaction, and week as fixed effects. All tests were two-tailed and a $p < 0.05$ was considered as statistically significant.

The power calculation was based on the predicted change of 20 % in 2-h plasma glucose level. Based on previous study [2] a sample of 12 subjects in a cross-over design provides sufficient power (95%) to detect the predicted change at a 5% significance level. A total of seventeen subjects were recruited to allow for drop out/non-compliance.

Results

Of seventeen subjects commencing the study, four subjects dropped out after completing the first part (2 subjects from each group), because of the time involved or starting the medication for treatment of hyperglycemia as advised by their general practitioner. Data of another subject were excluded later, due to her lack of compliance during the study. Thus, data of 12 subjects (5 males and 7 females) consisting of 10 cases of IGT and 2 new cases of T2DM with mean (\pm SD) age of 57.16 ± 12.88 years and BMI 28.85 ± 4.43 kg/m² were used for the final analysis. All 12 subjects received both placebo and supplement capsules in a cross-over manner. There was no significant difference between metabolic characteristics of subjects in placebo and supplement arms at the baseline (Table 1). No adverse effect was reported following consumption of 300 mg/d thiamine supplement or prepared placebo during the intervention period. All subjects had a compliance rate of at least 88% for the offered treatments.

Effects of high dose thiamine supplementation Six weeks of thiamine supplementation resulted in significantly increased RBC thiamine compared with baseline ($p < 0.001$). There was no significant change in RBC thiamine of subjects consuming placebo ($p = 0.94$) (Table 1). Following supplementation with thiamine, 2-h plasma glucose decreased significantly from $9.89(\pm 2.50)$ mmol/l at baseline to $8.78(\pm 2.20)$ mmol/l at week six ($p = 0.004$); no significant difference was detected in the subjects' 2-h plasma glucose concentrations when they received placebo (Figure 1). Fasting plasma glucose and insulin, and HOMA score at week six were significantly higher than those at baseline ($p = 0.003$, $p = 0.04$ and $p = 0.02$, respectively) in the placebo arm, but not in the supplement arm (Table 1). The mean 2-h plasma insulin measured at baseline and week six were not significantly different in either placebo (61.35 ± 44.07 vs. 68.34 ± 52.58 μ IU/mL, $p = 0.37$) or supplement (57.49 ± 41.66 vs. 64.88 ± 49.92 μ IU/mL, $p = 0.35$) arms (Figure 1). There was no significant change in renal function marker within or between arms.

Discussion

In the present study, supplementation with high dose thiamine for six weeks improved glucose tolerance significantly, as judged by 2-h plasma glucose. This finding is important from a clinical prospective. An elevation in 2-h blood glucose of 1 mmol/l can increase the risk of stroke-related death and all-cause mortality significantly [4]. Under hyperglycemic conditions, high dose thiamine has been suggested to increase transketolase activity, leading to a shift in the excess levels of metabolic intermediates from the glycolytic pathway toward the reductive pentose shunt [1]. This results in alleviating pressure on several pathways involved in hyperglycemia-induced complications as well as facilitating the glucose utilisation. Thiamine therapy for six weeks was shown to resolve glucose intolerance in a mouse model of TRMA in which the resultant diabetes mellitus was associated with decreased insulin secretion [5]. This suggests that, apart from the activation of thiamine-dependent enzymes and increased glucose utilisation, improvement of insulin secretion may also contribute to the beneficial effect of thiamine on glycaemic control [1]. This would help explain why thiamine supplementation appears to be more effective in those who still have insulin secretory capacity [2,5] compared with those pancreatic functions are destroyed [6,1]. It has been shown that 2-h plasma glucose is mainly determined by the postprandial insulin concentration at 30 minutes [7], reflecting the response of beta cells to the ingested glucose load. This measurement was not undertaken in the present study. Therefore, although in this study no significant change was observed in 2-h plasma insulin levels, it is not possible to judge that thiamine had no effect on insulin secretion. Further research focussing on the effect of high dose thiamine on insulin secretion by measuring postprandial plasma insulin and C-peptide levels every 30 minutes would be informative.

The present study indicated that in subjects receiving placebo, fasting plasma glucose and insulin, and HOMA-IR increased significantly from baseline after six weeks. However, there was no significant change in these measurements in the supplement arm. Recently, supplementation with 150 mg thiamine for one month was shown to decrease the fasting plasma glucose of diabetic patients with HbA1C level < 8%, who were not treated with medications [8]. These findings are inconsistent with the results of previous study [9] showing no significant change in the fasting glucose of diabetic subjects receiving high dose thiamine for 3 months. In that study, subjects had persistent microalbuminuria, and had been diagnosed with diabetes for at least 5 years. These results suggest that thiamine therapy may be less effective on the fasting plasma glucose of patients with long-term diabetes mellitus.

In conclusion, the current study suggests that thiamine supplementation may improve glucose tolerance in individuals with hyperglycemia at early stages, and may have a role in the prevention or delay of T2DM in patients with impaired glucose regulation. Further studies are required to confirm these results and investigate the mechanisms of these effects.

Duality of interest On behalf of all authors, the corresponding author states that there is no conflict of interest. This study was funded by an intramural grant.

Contribution statement J.S, M.J.S and F.A.S were involved in the conception and design of the study, and contributed to the final version of the manuscript. Data were collected, analysed and interpreted by F.A.S, who also drafted the manuscript. Y.Z provided the support with statistical analysis. All authors approved the final version to be published.

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Table 1 Fasting glucose and insulin, hemostatic model assessment, renal markers and thiamine status measured in clinical visits in thiamine and placebo arms

	Placebo arm (<i>n</i> =12)			Thiamine arm (<i>n</i> =12)			Between arms <i>p</i> - value
	Week 0 Mean (SD)	Week 3 Mean (SD)	Week 6 Mean (SD)	Week 0 Mean (SD)	Week 3 Mean (SD)	Week 6 Mean (SD)	
BMI (kg/m ²)	28.74(4.39)	28.89 (4.35)	28.87 (4.35)	28.88(4.25)	29.31 (4.11)	28.80 (4.23)	0.18
RBC TPP (nmol/g Hb)	0.95 (0.17)	-	0.95 (0.16)	0.93 (0.17)	-	1.56 (0.31) ^c	<0.001
Fasting glucose (mmol/l)	5.87(0.63)	5.87(0.77)	6.11(0.70) ^b	6.02(0.68)	6.03(0.70)	6.01(0.80)	0.009
Fasting insulin (μIU/ml)	6.64(3.45)	7.17(3.50)	7.67(4.39) ^a	7.36(4.41)	7.53(4.21)	7.46(4.67)	0.031
HOMA score	1.75(1.01)	1.89(1.07)	2.09(1.32) ^a	2.00(1.31)	2.04(1.27)	2.05(1.51)	0.01
eGFR (ml min ⁻¹ 1.73 m ⁻²)	97.5 (31.7)	98.9 (28.4)	98.3 (28.3)	96.0 (25.8)	97.9(29.2)	95.1 (25.1)	0.49

Plasma glucose was measured with the hexokinase method. Plasma insulin was determined by the Architect insulin assay, using the Abbott diagnostic kits (Abbott Laboratories, IL, and USA). eGFR (estimating glomerular filtration rate) was determined by Cockcroft-Gault formula adjusted for body surface area. RBC thiamine pyrophosphate (TPP) was determined by high-performance liquid chromatography (HPLC) with fluorescent detection (pre-column derivatisation) using the Chromsystems reagent kit (Chromsystems Instruments and Chemicals GmbH, Munich, Germany) validated for RBC samples. ^a *p*<0.05, ^b *p*<0.01, ^c *p*<0.001 compared with baseline.

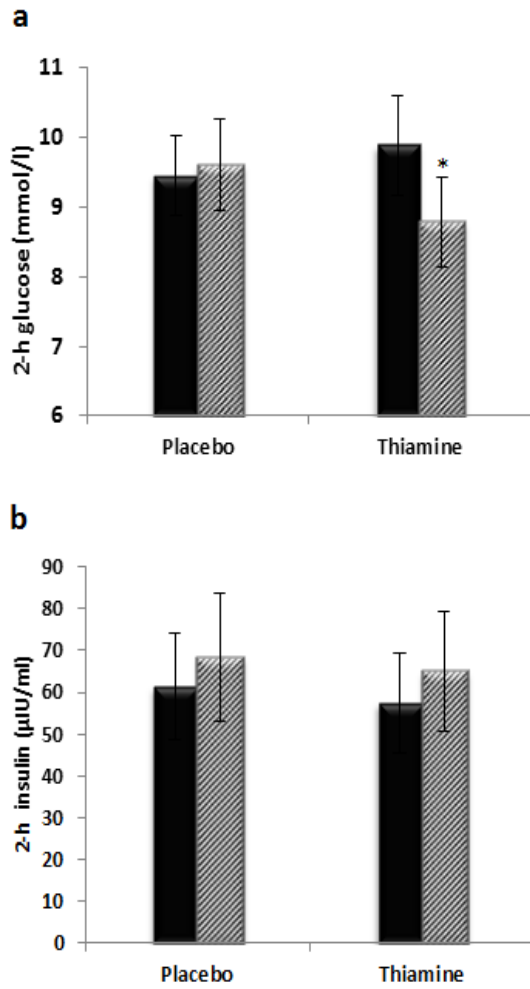


Fig. 1 Effects of high dose thiamine on 2-h plasma glucose and insulin. 2-h plasma glucose (a) and insulin (b) measured at baseline and week 6 in thiamine and placebo arms. Data are presented as mean±SEM. *, $p < 0.01$ compared to baseline; ■, week 0; ▨, week 6.