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Hyperglycemia induces elevated expression of thyroid hormone binding protein *in vivo* in kidney and heart and *in vitro* in mesangial cells

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ABSTRACT

During a search for glucose-regulated abundant mRNAs in the diabetic rat kidney, we cloned thyroid hormone binding protein (THBP), also known as μ-crystallin or CRYM. The aim of this study was to investigate the effect of hyperglycemia/high glucose on the expression of THBP. THBP mRNA copy numbers were determined in kidneys and hearts of diabetic GK rats vs normoglycemic Wistar rats, and in human mesangial cells (HMCs) exposed to high glucose using real-time qPCR, and THBP protein levels were measured by Western blotting and immunofluorescence. Intracellular ROS was measured in THBP transfected cells using DCF fluorescence. Hyperglycemia significantly increased THBP mRNA in GK rat kidneys (326 ± 50 vs 147 ± 54, $p < 0.05$), and hearts (1583 ± 277 vs 191 ± 63, $p < 0.05$). Moreover, the levels of THBP mRNA increased with age and hyperglycemia in GK rat kidneys, whereas in normoglycemic Wistar rat kidneys there was a decline with age. High glucose significantly increased THBP mRNA (92 ± 37 vs 18 ± 4, $p < 0.005$), and protein in HMCs. The expression of THBP as a fusion protein in transfected HMCs resulted in reduction of glucose-induced intracellular ROS. We have shown that THBP mRNA is increased in diabetic kidney and heart, is regulated by high glucose in renal cells, and appears to attenuate glucose-induced intracellular ROS. These data suggest that THBP may be involved in the cellular pathways activated in response to glucose. This is the first report linking hyperglycemia with THBP and suggests that the role of THBP in diabetic complications should be further investigated.

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Introduction

Diabetic nephropathy (DN) is a progressive renal disease which affects 30–50% of patients with diabetes mellitus and is the major cause of end stage renal failure in the Western World [1]. Hyperglycemia leads to increased cytosolic glucose concentrations in renal cells with consequent biochemical dysfunction such as the abnormal activation of various signaling pathways and oxidative stress [2–4]. Diabetic cardiovascular and renal diseases share many of the same risk factors and renal disease is accompanied by an increased global cardiovascular risk [5]. The exact molecular mechanisms which result in diabetic complications are not fully understood.

We cloned thyroid hormone binding protein (THBP) as one of several candidate differentially expressed gene from diabetic rat kidneys which are induced in response to hyperglycemia [6,7]. Also known μ-crystallin or CRYM, THBP was originally proposed to be a major structural protein in the lens with possible enzymatic functions due to its structural homology to bacterial enzymes alanine dehydrogenase and ornithine cyclodeaminase [8], and has since been shown to be a cytosolic protein involved in the intracellular transport of thyroid hormone [9].

The objective of this work was to test the hypothesis that hyperglycemia can affect the expression levels of THBP. We used kidneys and hearts from the GK rat, a spontaneous model of Type 2 diabetes [18,19] at different ages, to examine the effect of hyperglycemia on renal and cardiac THBP expression. GK rats are normoglycemic at birth but spontaneously develop hyperglycemia being diabetic by 26 weeks of age. These rats were originally derived from Wistar rats by selective breeding, therefore comparison of GK rats with Wistar rats, as well as comparison of GK rats before and after the spontaneous development of hyperglycemia can allow for evaluation of the *in vivo* effects of hyperglycemia on gene expression [20]. We also used cultured human mesangial cells to examine the direct effect of high glucose on THBP. Mesangial cells are one of the major sites of glomerular damage in the diabetic

Abbreviations: THBP, thyroid hormone binding protein/μ-crystallin; CRYM, crystallin M; ROS, reactive oxygen species; DN, diabetic nephropathy; HG, high glucose/25 mM glucose; NG, normal glucose/5 mM glucose; TBHP, *tert*-butyl hydrogen peroxide; DCF, 5-carboxy-2′,7′-dichlorodihydrofluorescein diacetate/H_2DCFDA

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Table 1
Oligonucleotide primers used in this study.

Gene (Accession No.)	Primer	Oligonucleotide sequence (5′–3′)	Product size (bp)	Annealing temp/mM $MgCl_2$
Rat THBP/Y17328	rTHBP F1	TCA GTG CAA GGA GAT GTT CG	189	58/3
	rTHBP R1	TGG ATG ACG AGC TCA TGA AG		
Human THBP/L02950	hTHBP F1	GAA GCT GTG CTG TAC GTG GA	185	62/3
	hTHBP R1	GAA GAC ACA GT T GCA GCC AA		
Human THBP/BC018061	hTHBP F5	GGT GCG GAG ACT GAG GTT AG	920	57/1
	hTHBP R5	GA AGA CAC AGT TGC AGC CAA		
Rat β-actin/NM_031144	rβ-actin F	ACG GTC AGG TCA TCA CTA TC	299	55/3
	rβ-actin R	AGC CAC CAA TCC ACA CAG A		
Human β-actin/NM_001101	hβ-actin F	TGT GCC CAT CTA CGA GGG GTA TGC	433	55/3
	hβ-actin R	GGT ACA TGG TGG TGC CGC CAG ACA		

kidney and have been widely used as models to study diabetic nephropathy [21–23]. Lastly we undertook studies to examine the role of THBP expression on the cells' oxidative stress response by measuring intracellular ROS in the cell.

Table 2
Relative copy numbers of THBP mRNA in GK and Wistar rat kidneys at 6 and 26 weeks.

Tissue	Rat model	THBP mRNA copy numbers relative to 1000 β-actin copies, mean values ± SD, $n = 2$	
		Age 6 weeks	Age 26 weeks
Kidney	GK	64 (±10)	326 (±50)
	Wistar	271 (±59)	147 (±54)
Heart	GK	775 (±123)	1583 (±277)
	Wistar	686 (±38)	191 (±63)

Materials and methods

Biological material. GK and Wistar rat kidneys and hearts at defined ages were obtained from the University Hospital of Wales, Biomedical Services Institute in collaboration with Professor John Williams (Institute of Nephrology, College of Medicine, University of Wales,) under local ethical rules [24]. Human mesangial cells were grown as previously described [21,22]. Human tissue RNAs were obtained as a commercial panel (Ambion, UK).

Preparation of RNA and cDNA. Total RNA was extracted from rat tissues using the Total RNA kit (Ambion) or from cells using RNAqueous-4PCR kit (Ambion, UK) and cDNA was prepared as previously described [21,22].

Quantitative real-time PCR. Real-time qPCR was carried out in a total volume of 10 μl containing forward and reverse primers, FastStart DNA Master SYBR Green^{plus} (Roche Molecular Biochemicals), DNA template and nuclease free water in the Roche LightCycler

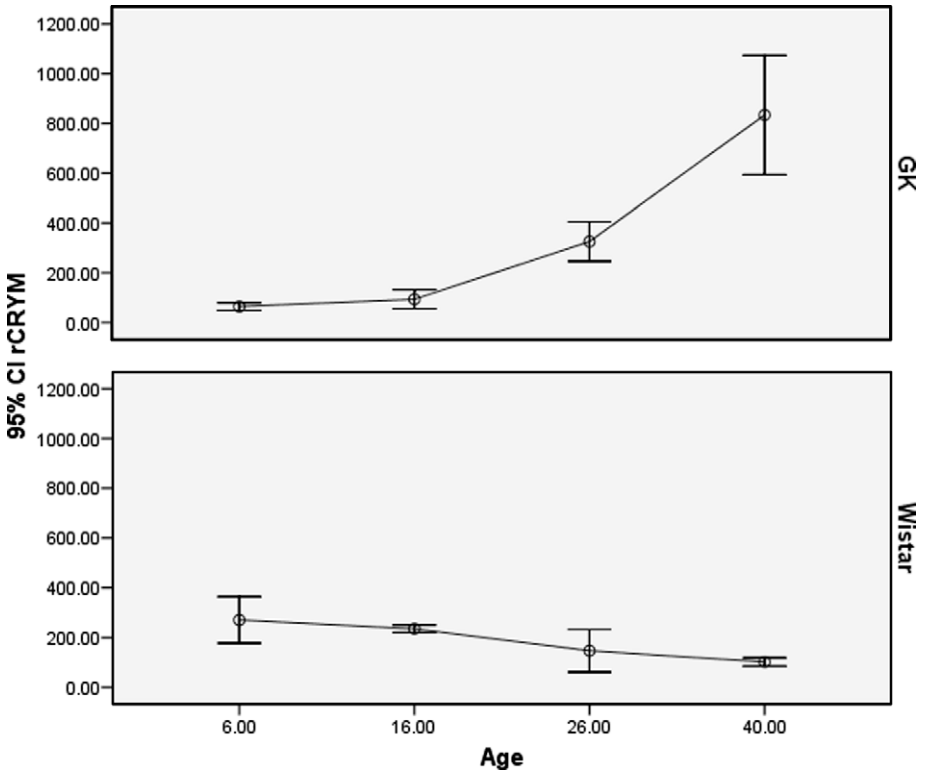

Fig. 1. THBP mRNA copy numbers in GK and Wistar rat kidneys at progressive stages. Kidneys from progressively hyperglycemic GK rats and age-matched normoglycemic Wistar rats (W) were used to quantify THBP mRNA by real-time qPCR. Each data point is shown on the scatter plot with 95% confidence interval. There is a highly statistically significant difference between GK 6 and 26, GK 6 and 40, GK 16 and 26 and GK 16 and 40 ($p < 0.005$) but no significant difference between GK 6 and 16 ($p > 0.005$). In Wistar kidneys there is a highly significant difference between W 16 and 40, whilst all other stages show <0.005 significance, and no significant difference between W 6 and 16 ($p > 0.005$).

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