Mammal Cells Double Their Total RNAs against Diabetes, Ischemia Reperfusion and Malaria-Induced Oxidative Stress

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Supplemental material

Table SM-1. List of primers for characterizing human genes.

Gene	Location (map)	L primer	R primer
Actin1	NM_001100	CCTGACCCTGAAGTACCCTA	GTGATGACCTGCCCGTCT
UBC	NM_021009	CAAAGATCCAGGACAAGG	TCTAAGACGGAGCACCAG
Hxk1 Line 1	NM_000188	TGGAGTCCGAGGTTTATG	GATGCAGGAGACAATGTGA
Hxk1-Anti Line 1	NM_000188	TGCTCACCCGAGGGAAGT	TCAATAGGAATGGCGTAGA
Hxk1 Line 2	NM_000188	GCTCACCCGAGGGAAGTA	TCTGCTGGCAGGGAAATG
Hxk1-Anti Line 2	NM_000188	TCAGTCCAGCACGTTTGC	GAGCCAGGGTCTCCTCTAT
Hxk2 Line 1	NM_000189	ATGGACCAAGGGATTCAA	TCTGTGCGGAAGTCATCTAG
Hxk2-Anti Line 1	NM_000189	CCCGCCAGAAGACATTAG	AACCACATCCAGGTCAAAC
Hxk2 Line 2	NM_000189	GTGGTGGACAGGATACGA	CTGACTGCCCTAAGAATAAA
Hxk2-Anti Line 2	NM_000189	CTGGACAGCGATAGAACC	ATGGAATACTGCCAAGAAA
Hxk3 Line 1	NM_002115	ACATGGCACTGAGCAAGGA	ACATGGGAAGGAGAAGGTAAA
Hxk3-Anti Line 1	NM_002115	GGGGCTTCGGATGTTGAG	CCACAGTCTCGGGAATGGA
Hxk3 Line 2	NM_002115	TGTGAGGTTGGGCTAGTTGT	CTGCGAGTGATGGCTTCC
Hxk3-Anti Line 2	NM_002115	CTTCGGATGTTGAGCTTGTG	CGCAGTCTGATGCCTTGA
TBP	NM_003194	CTGCCACCTTACGCTCAG	CCTTTAGAATAGGGTAGATGTT
TBP-Anti	NM_003194	CACTCCACTGTATCCCTCC	CTCTGGCTCATAACTACTAAAT
TFIIA-1	NM_015859	ATACAAACACCGTGCCTAA	TTCTTCCACCTGCCCATC
TFIIA-2	NM_004492	AGAGGGTCAGGAACAGAG	TACCATCACAGGCTACAA
TFIIB	NM_001514	TCGGAGAACAATGAGCAG	ACATCAGCAACACCAGCA
TFII-I	NM_032999	AGGGCAATGAAGGCACAG	CCAGGAGGCAAGTAGGAA
CBP (CREBBP)	NM_004380	GTCTGCCTTCTCCTACCTCA	GCCTCCGTAACATTTCTCG
P300	NM_001429	GGAGGCACTTTACCGTCAG	GGGCAGTCAGAGCCATAC
PRDX1	NM_002574	TGGTGTCGGTGGTTAGTT	CCCAGTCCTCCTTGTTTC
SOD	NM_000454	GCTGGTTTGCGTCGTAGT	CTTCATTTCCACCTTTGC
CAT	NM_001752	GTTGAAGATGCGGCGAGAC	GGGCAGAAGGCTGTTGTT
GPX	NM_000581	CAACCAGTTTGGGCATCAG	CCGTTCACCTCGCACTTC
chr6.trna160-AlaAGC	Chromosome 6	GGGGAATTGGCTCAAGCG	GCGTCGATCCTGCTACCT
chr1.trna113-AsnGTT	Chromosome 1	CTGTGGCGCAATCGGTTA	TGGGCTCGAACCACTAAC
chr7.trna19-CysGCA	Chromosome 7	GGGGGTATAGCTCACAGG	AGGGAGTAACCGGATTTG
chr6.trna88-PheGAA	Chromosome 6	GCCAAAATAGCTCAGCTG	TTCTGAAACCCAGGATCA
5S rRNA	NR_023363	CTACGGCCATACCACCCT	GGTATTCCCAGGCGGTCT
5.8S rRNA	NR_003285	CTTAGCGGTGGATCACTCG	AAGCGACGCTCAGACAGG
28S rRNA	NR_003287	TTCGGGATAAGGATTGGCTCTA	GGCTGTGGTTTCGCTGGAT
18S rRNA	NR_003286	TCCTTTGGTCGCTCGCTCCT	TCGCTCTGGTCCGTCTTGC

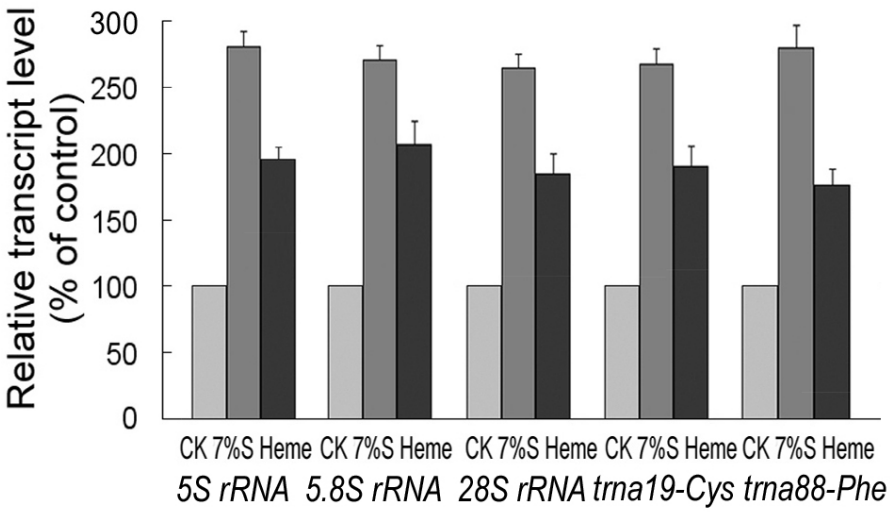

Figure SM-1. Effects of 7% glucose (7%S) and 50 μM heme treatments (24 h) on *5S rRNA*, *5.8S rRNA* and *28S rRNA* (three representative rRNAs), and *trna19-Cys* and *trna88-Phe* (two representative tRNAs) expression. Gene expressing detection was derived on an equal DNA basis. Error bars show standard deviations (*n*=3).

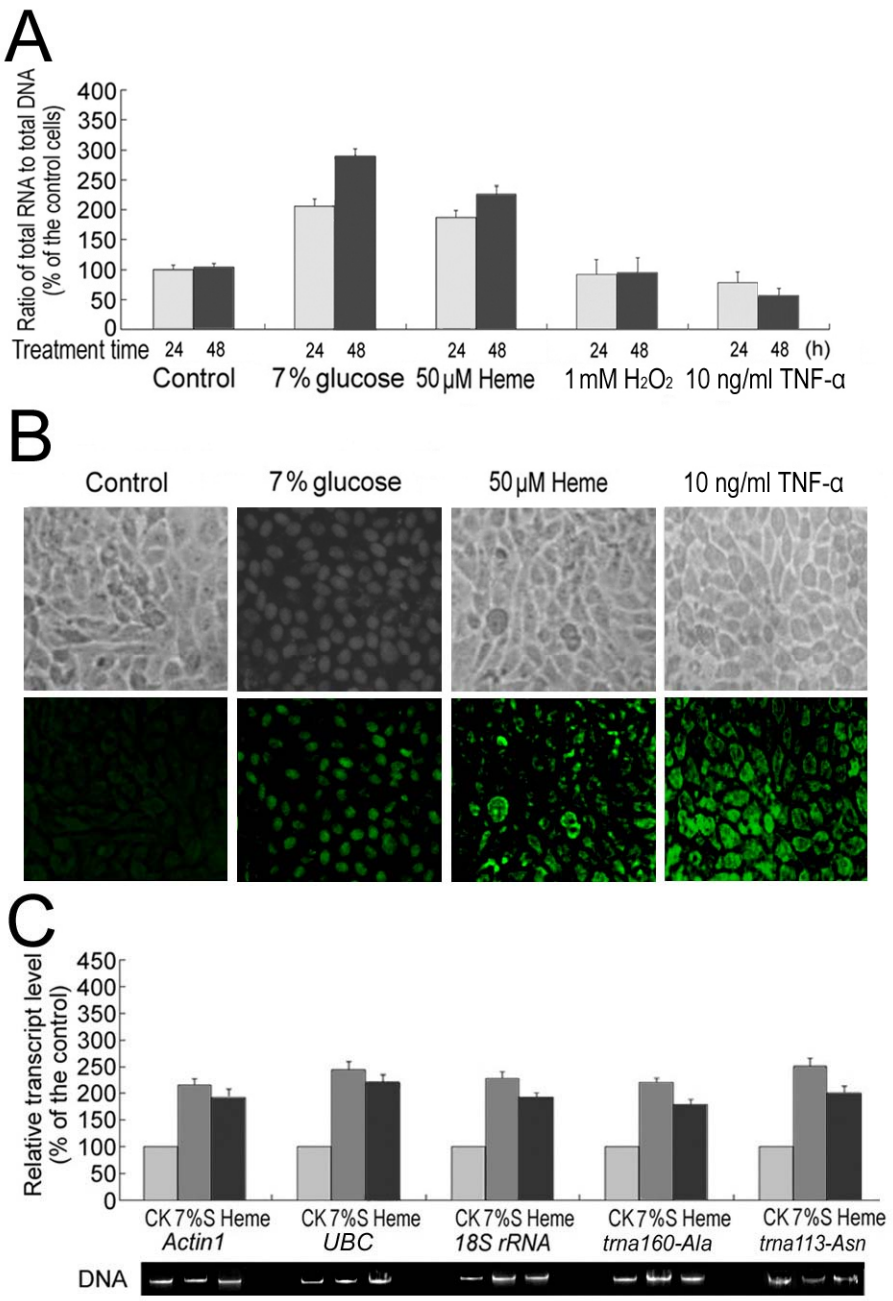

Figure SM-2. Glucose and heme induce RNA amplification in Chang liver cells. (A) 7% glucose or 50 μM heme but not 1 mM H_2O_2 or 10 ng/ml TNF-α doubles Chang liver cell total RNAs within 24 h. (B) Cell forms (upper panel) and H_2O_2 levels (lower panel) of heme, glucose or TNF-α-treated cells (24 h). H_2O_2 was visualized by CM-DCFH-DA stain and observed with a fluorescence microscopy. (C) Effects of 7% glucose (7%S) and 50 μM heme treatments (24 h) on *Actin1* and *UBC* (two representative mRNA), *trna160-Ala* and *trna113-Asn* (two representative tRNAs) and *18S rRNA* expression. Gene expressing detection was derived on an equal DNA basis. Error bars show standard deviations (*n*=3).

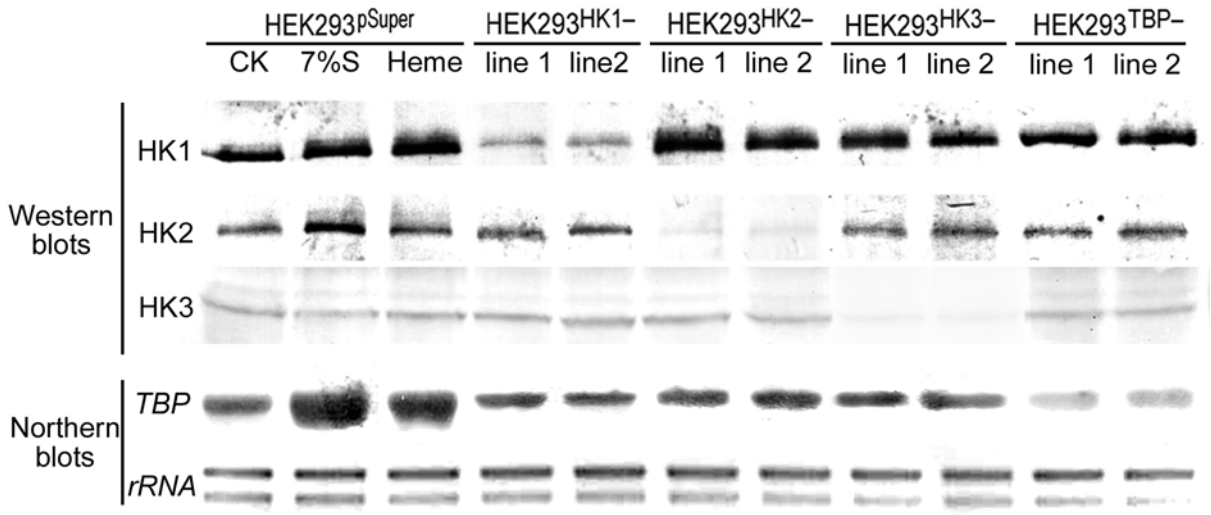

Figure SM-3. (C) Effects of 7% glucose (7%S) and 50 μM heme on HK1, HK2, HK3 protein levels and *TBP* gene expression. Hexokinase protein levels were detected by Western blotting. *TBP* expression was detected by Northern blotting. SYBR green I-stained *rRNA* is shown as a loading control. Two independent gene-silenced cell lines for each gene were tested. Error bars show standard deviations (*n*=3).

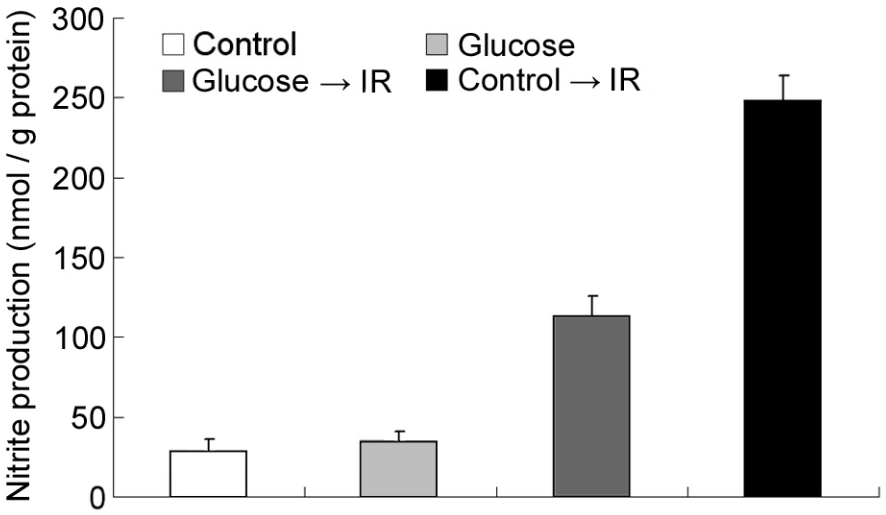

Figure SM-4. Effect of glucose infusion and ischemia-reperfusion on NO production. The rat kidneys were treated with or without 30% glucose (renal arterial infusion), and then IR was performed. Kidney samples were taken at 24 h after the glucose infusion or 24 h after reperfusion. Error bars show standard deviations ($n = 3$).

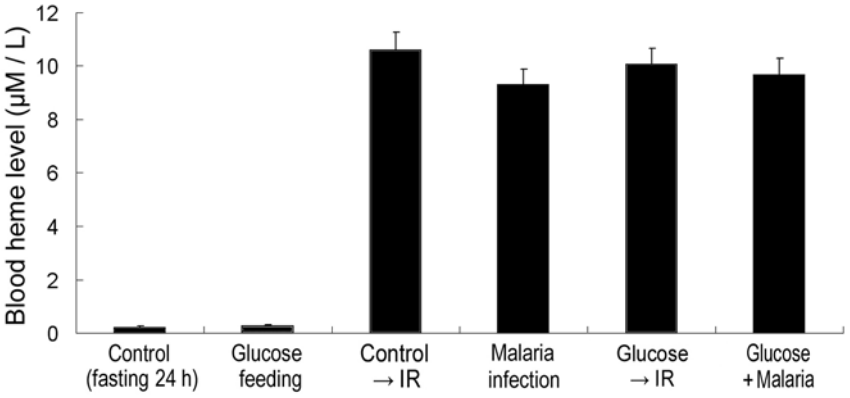

Fig. SM-5. Blood free heme levels after glucose feeding (to diabetic rats), ischemia reperfusion (IR) and malaria infection. The rat (fasted for 24 h for the "Control" sample to diabetic experiments) kidneys were treated with or without 30% glucose (once for IR experiments; every two days accompanying with malaria infection), and then 30% glucose solution was administered orally (to diabetic animals, every 8 hours, three times total), or IR or malaria infection (to normal animals) was performed. Blood samples were taken at 24 h after the first glucose administration or 24 h after reperfusion, or 8 days after malaria infection. Error bars show standard deviations (n = 3).

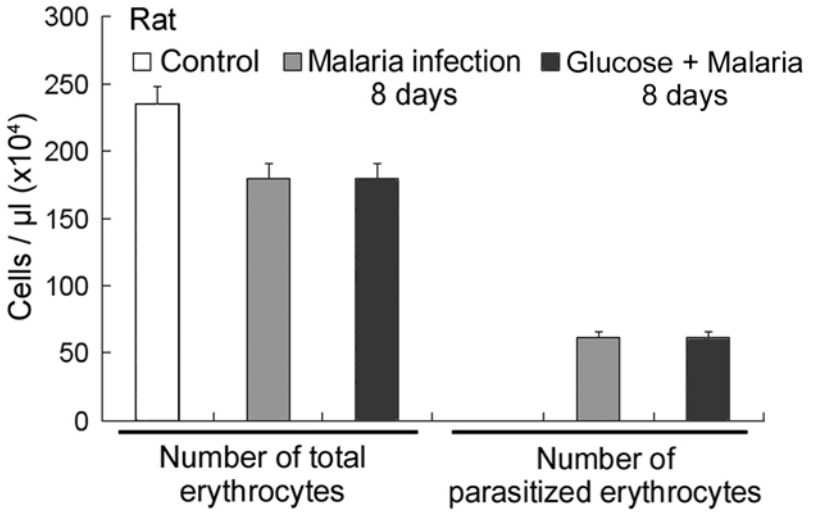

Figure SM-6. Severity of malaria infection to rats. Malaria infection was applied with 10^6 *Plasmodium berghei* to normal rats. 8 days later, the number of erythrocytes/µl renal blood ($\times 10^4$) and the number of parasitized erythrocytes/µl renal blood ($\times 10^4$) were examined by a microscopy. Error bars show standard deviations (n = 3). For alleviating malaria infection, rat kidneys were treated with 30% glucose solution (renal arterial infusion, every two days, four times total) paralleling with *Plasmodium berghei* infection for 8 days.

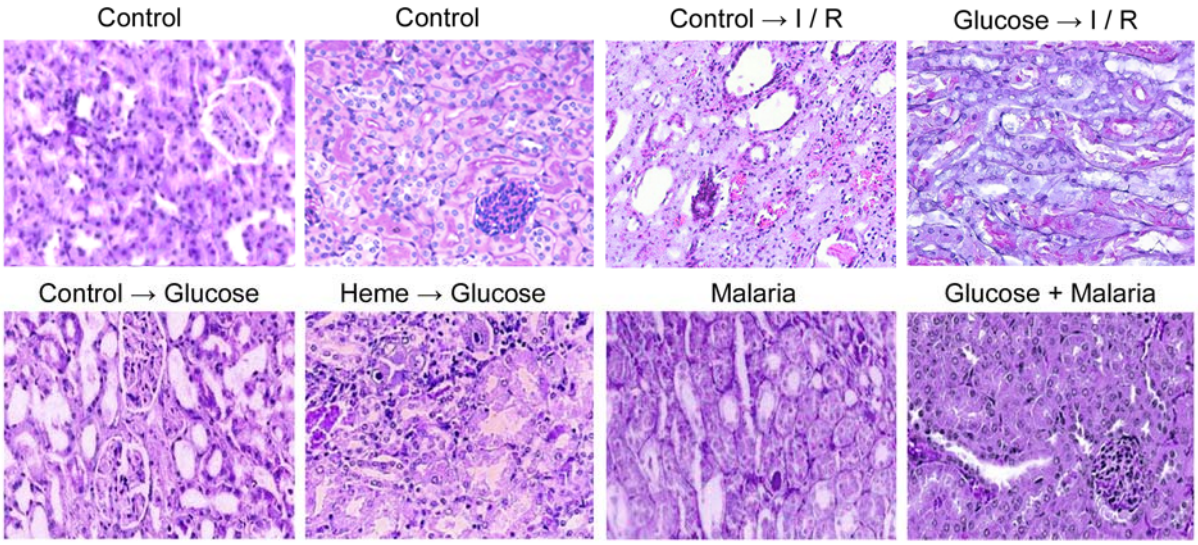

Figure SM-7. Subcortical histological sections of kidneys stained with periodic acid-schiff from groups studied as stated. Tubular dilation and loss of brush border occur and flattened epithelial cells are found when the rats were subjected to diabetes, ischemia reperfusion (I/R) or malaria infection. See the legend of Fig. 5 and "Material and Methods" for details.

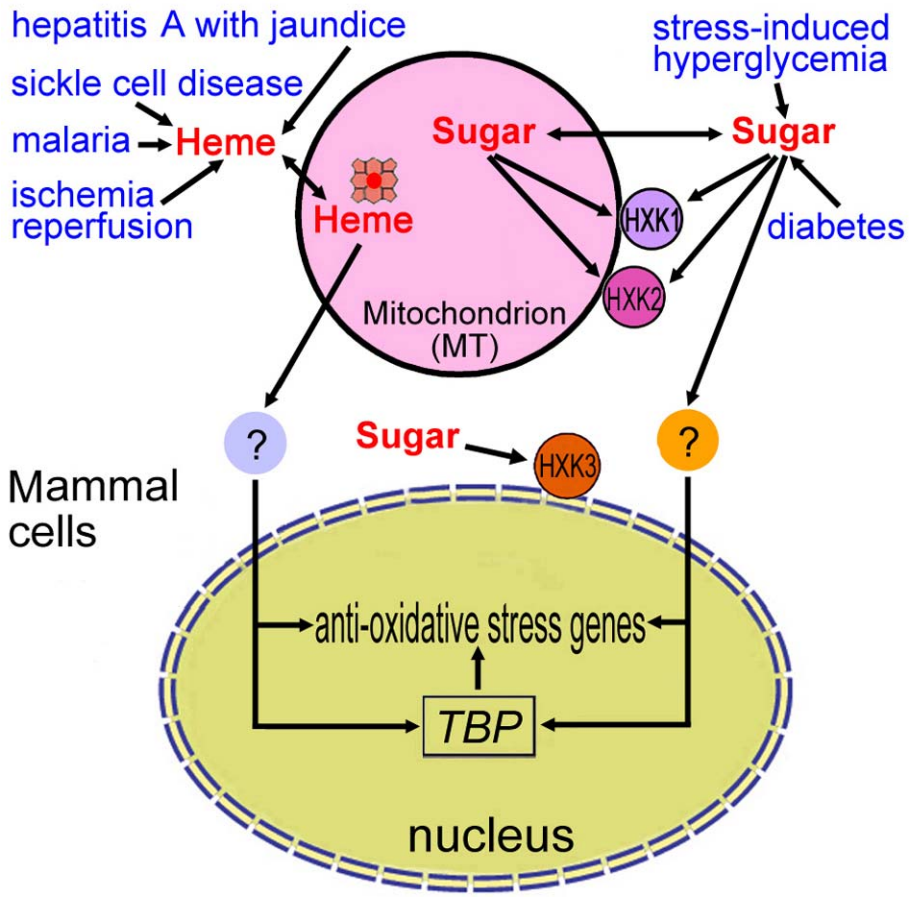

Figure SM-8. Model of heme and sugar signals regulating cellular total RNAs and oxidative stress resistance. For mammal cells, stress-induced hyperglycaemia (such as head injury or acute stroke) and diabetes increased blood glucose substantially, and the subsequent ROS. Sickle cell disease, ischemia reperfusion, icterohepatitis and malaria results in high levels of free heme, also causing undesirably oxidative stress. Under these two circumstances, *TBP* (encoding the TATA box-binding protein) transcript is promoted, and subsequently cellular total RNAs are increased. Anti-oxidative stress gene expression is also promoted simultaneously. Hexokinase does not mediate sugar signaling in mammal cells. However, sugars generate ROS in mammal cells depending on mitochondrial-bound hexokinase 1 and 2. Human hexokinase 3 has a perinuclear localization.