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Original Article

High glucose concentration restricts fat consumption in *Caenorhabditis elegans*

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Abstract: This study investigated the effects of high glucose levels on physiological function and lipid accumulation of *Caenorhabditis elegans (C. elegans)*. *C. elegans* was incubated with 100 mM glucose for 72 h. Physiological function, fat content and metabolic enzyme expression were tested to determine the effects of high glucose exposure. As a result, *C. elegans* treated with 100 mM glucose showed shorter body length and decreased production of offspring compared with the worms exposed to normal diet, while no changes in pumping or locomotion were observed. Oil-Red-O (ORO) staining showed increased fat content in *C. elegans* exposed to high glucose concentration, which suggests defective energy utilization. Considering the importance of enzymes in energy expenditure, the gene expression of 44 enzymes involved in glucose and lipid metabolic pathways were tested using quantitative real-time polymerase chain reaction (qRT-PCR). The results showed that high-glucose treatment affected the gene expression of 10 glucolipid enzymes. A dramatic decline in three enzymes (*acs-15, acs-18, acdh-8*) involved in mitochondrial beta-oxidation of fatty acids suggests inhibition of fat breakdown. In conclusion, high glucose concentrations induce physiological defects in *C. elegans*, which might be attributed to impaired fatty acid metabolism in mitochondria.

Keywords: *Caenorhabditis elegans (C. elegans)*, high glucose cultivation, mitochondrial beta-oxidation

Introduction

Obesity is a significant risk factor for hypertension, diabetes, coronary heart disease and cancer [1-3]. However, the prevalence of obesity has increased in many countries over the past four decades, and has turned into a public health crisis, resulting in huge economic burden to the society. Current trends suggest that by 2025, the prevalence of obesity may climb to 18% in men and surpass 21% in women globally [4].

Chronic imbalance in energy intake and consumption may affect lipid metabolism [5]. Diet is a major source of energy intake, especially the high intake of sugar or sugar-sweetened beverages is closely linked to obesity. Glucose derived from dietary sugar is crucial not only for fat accumulation but also for development, fertility, and life span of organisms [6].

In this study, we investigated the effect of high glucose concentration on specific physiological functions and fat content of *Caenorhabditis elegans* (*C. elegans*). Changes in the expression of metabolic enzymes were studied to elucidate the potential mechanisms of high glucose-mediated energy metabolism.

Materials and methods

Strains

Wild type N2 Bristol and *Escherichia coli* OP50 were provided by the Caenorhabditis Genetics Center funded by the National Institutes of Health (NIH) National Center for Research Resources (NCRR).

Culture and glucose treatment

Eggs of *C. elegans* were obtained by treating gravid adults with hypochlorite-NaOH. Embryos were allowed to develop in M9 buffer at room temperature (RT) overnight. Batches of L1 larvae obtained on the next day were transferred to nematode growth medium (NGM) agar plates seeded with *Escherichia coli* OP50 at 20°C. According to previous study, NGM agar plates were added with or without 100 mM glucose

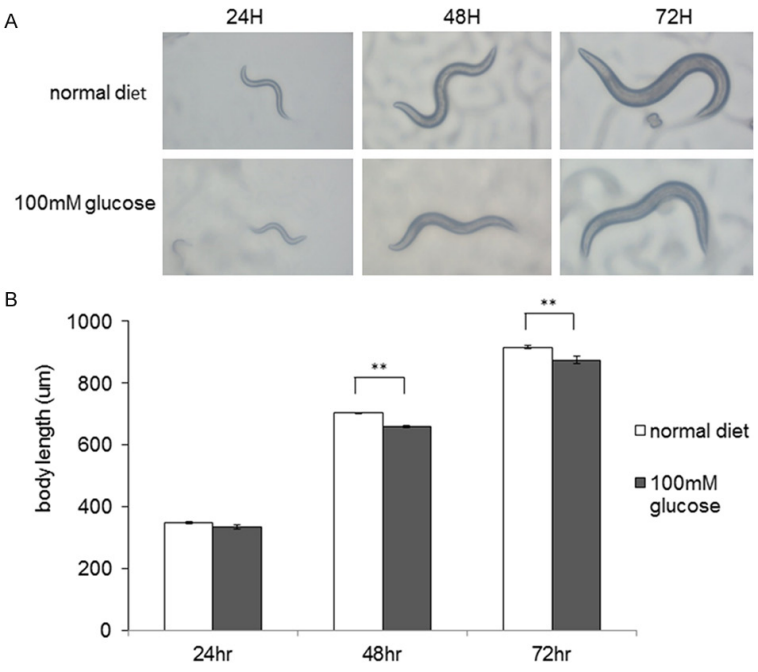

Figure 1. Effect of high glucose concentration on body length of *C. elegans*. A. Morphology of *C. elegans* fed with or without 100 mM glucose for 24 h, 48 h and 72 h. B. Body length revealed a decline in the development of worms fed on extra 100 mM glucose after 48 h and 72 h of cultivation ($P<0.01$). The error bars represent standard deviations. **$P<0.01$.

[7]. L1 larvae developed to L4 stage after 48 h, and into young adults after 72 h.

Body length measurement

Fifteen worms in each group were placed on NGM agar plates. Each animal was imaged using the Olympus IX51 (Olympus Corporation, Shinjuku, Japan) and measured along the central axis of the nematode from head to tail after cultivating L1 larvae for 24 h, 48 h and 72 h. The average length of 15 worms was obtained.

Locomotion

L1 larvae developing into L4 stage were transferred to a new NGM plate and allowed to adapt for 10 min. The rate of locomotion was determined by calculating the number of body bends in each worm over a 20 s period, under a stereomicroscope. When the area immediately behind the pharynx reached a maximum bend in the direction opposite to the previous bend, the count was advanced by one [8]. At least ten worms in each group were studied.

Pharyngeal pumping

Each L4 larva was placed in a new NGM plate with a fairly thin lawn of OP50. The pumping rate was observed under a stereomicroscope, and measured by counting the grinding movement for 10 s. At least 10 worms in each group were measured and the average was calculated.

Fertility assays

Five young adults in each group starting ovulation were placed on NGM plates with or without glucose. After incubation for 5 h, the animals were removed and the number of eggs was measured. The ovulation rate was calculated as follows: number of eggs/[number of nematodes (5) × time (5 h)].

Oil-Red-O (ORO) staining

Oil-Red-O staining was conducted by collecting batches of worms with 1 × PBS. The cuticles of worms were permeabilized by rocking samples with 1 × PBS and 2 × MRWB buffer for 1 h at RT. Each sample was incubated in 60% isopropanol for 15 min at RT for dehydration. The worms were suspended in 1.0 mL of 60% ORO stain and incubated on the bench overnight at RT [8, 9]. After removal of ORO dye next morning, the stained worms were observed and imaged using Olympus IX51 (Olympus Corporation, Shinjuku, Japan).

ORO also stains eggs, which are high in fat content. Therefore, the body fat of worms in the L4 stage was estimated to avoid interference with the degree of ovulation in each adult.

ORO quantification

ORO stain was quantified by extracting the dye from 100 to 200 worms in each group with 10 to 20 μL isopropanol incubated at 37°C for 2 h [8]. The density of extraction at 490 nm with NanoDrop2000 spectrophotometer (ThermoScientific, Wilmington, DE, USA) was estimated using a standard curve representing

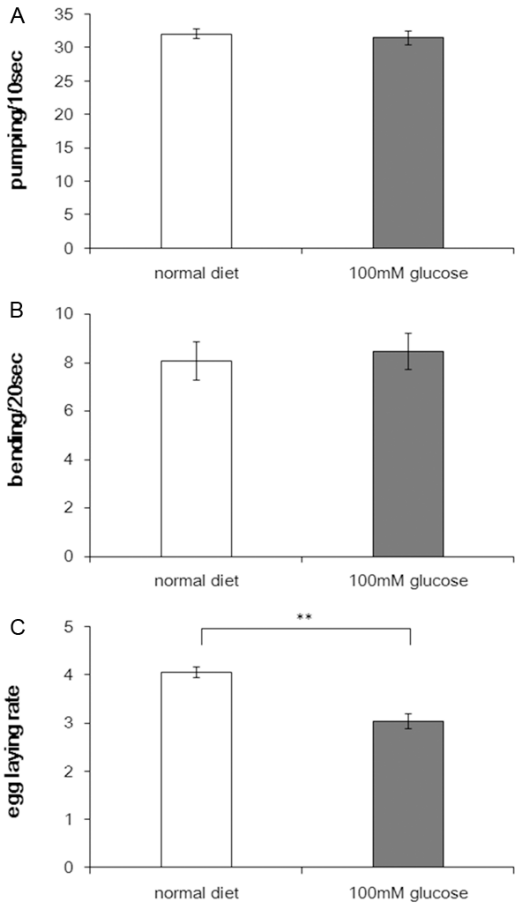

Figure 2. Effect of high glucose on physiological function of *C. elegans*. A. The degree of pharyngeal pumping of worms in 10 s showed no statistical difference between those fed on normal diet and those exposed to 100 mM glucose. B. No statistical difference was seen in the number of body bends of worms within 20 s of feeding with or without 100 mM glucose. C. The rate of ovulation in the 100 mM glucose group was lower than in the controls (P<0.01). The error bars represent standard deviations. **P<0.01.

different concentrations of ORO stain. Levels of ORO quantification of worms fed on 100 mM glucose were normalized to those exposed to normal diet.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Three independent replicates of each group were prepared. RNA was extracted from samples using the TRIzol method. The qRT-PCR reactions were conducted and analyzed on a 7900HT Fast Real-Time PCR System (Life Technologies, Grand Island, NY, USA) using FastStart Universal SYBR Green Master (Rox) (F. Hoffmann-La Roche Ltd., Basel, Switzerland). The complete set of genes and primers was designed according to the description in the Wormbase databank (http://www.wormbase.org/), and is listed in Supplementary Table 1 [8, 10].

Statistical analysis

All the calculations were analyzed using IBM SPSS. Experiments were performed in triplicate under similar conditions. Results were expressed as the means ± SD. Statistically differences were considered significant when the *p* value was less than 0.05.

Results

High-glucose concentration decreases the body length of C. elegans

To determine the effect of high-glucose concentration on the development of nematodes, we examined the body length on three consecutive days after transferring L1 larvae into the NGM plates with or without 100 mM glucose.

The results showed no differences in body length following exposure to high glucose levels for 24 h (**Figure 1A**, **1B**). However, the body length of nematodes was reduced following exposure to high-glucose media for 48 h and 72 h compared with normal media (*P*<0.01; **Figure 1A**, **1B**).

Effect of high-glucose diet on the physiology of C. elegans

The growth retardation suggested possible interference with energy intake or consumption by high glucose levels. Therefore, the pumping rate, locomotion and degree of ovulation were tested.

Pumping function directly affects the total energy intake of nematodes. Body bending is related to energy expenditure of worms. The frequency of pharyngeal pumping and body bending was monitored periodically. No significant differences in these two physiological functions were observed between control and high-glucose fed worms (**Figure 2A**, **2B**). Ovulation is one of the most energy-intensive activities of *C. elegans*. High-glucose diet

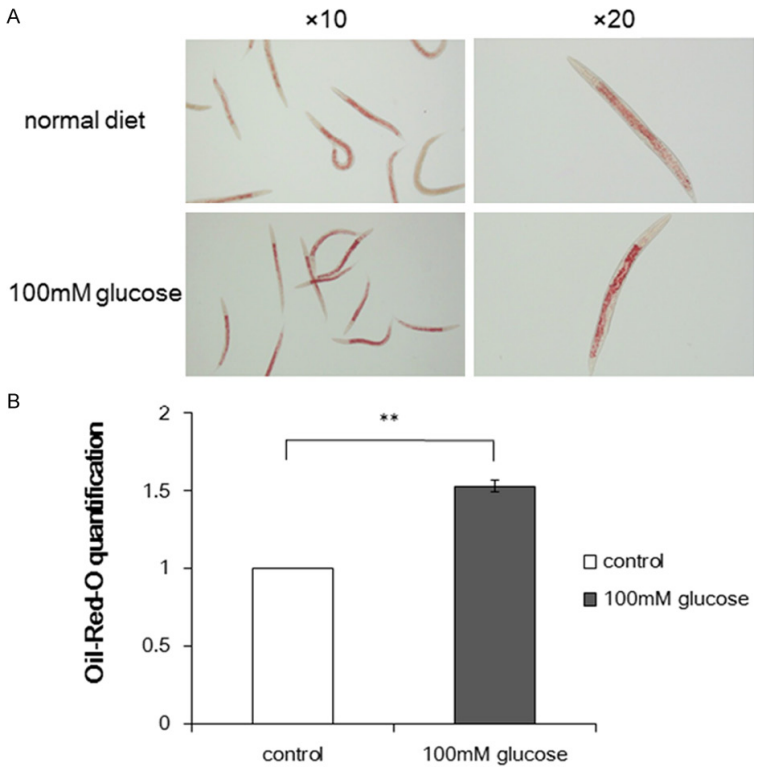

Figure 3. High glucose concentrations elevated the fat content of *C. elegans*. A. Images of lipid droplets dyed with Oil-Red-O (ORO) in nematodes fed with or without 100 mM glucose under × 10 and × 20 magnification. B. Exposure to 100 mM glucose increased the fat content of *C. elegans* (P<0.01). The error bars represent standard deviations. **P<0.01.

reduced the number of offspring compared with control animals (*P*<0.01; **Figure 2C**).

Elevated fat content in nematodes fed on high glucose

High-glucose diets decreased body length and degree of ovulation in worms. Therefore, the influence of glucose on fat accumulation in *C. elegans* was further investigated. We labeled the lipid droplets of nematodes with Oil-Red-O (ORO) dyes. Images showed that worms fed with high-glucose diet stained more brightly than the control worms (normal diet group) (**Figure 3A**). Results of the ORO quantification analysis were consistent with microscopic observations (*P*<0.01; **Figure 3B**) suggesting that high glucose levels increased fat accumulation in *C. elegans*.

High-glucose concentrations mainly affect gene expression in mitochondrial fatty acid beta-oxidation

Fat content was significantly increaed in high-glucose concentration, while the body development and ovulation of these nematodes declined. It suggested an impaired fatty acid breakdown. Lipid metabolism is regulated by several enzymes involved in energy metabolism. Therefore, we used qRT-PCR to elucidate the expression of 44 major genes involved in lipid and glucose metabolism in nematodes exposed to high glucose levels.

As a result, the transcription of 10 genes involved in mitochondrial fatty acid beta-oxidation (*acdh-2*↑, *acs-2*↑, *acdh-8*↓, *acs-15*↓, *acs-18*↓, *ech-1.1*↓, *R09E10.4*↓) and glucose metabolism (*gpd-1*↓, *gpd-4*↓, *pfk-1.2*↓) was altered. The expression of *acdh-8*, *acs-15* and *acs-18* showed dramatic changes (**Figure 4**; Supplementary Table 1).

Thus, high-glucose diet reduced the energy metabolism of *C. elegans* while a few enzymes showed a mild increase in expression.

Discussion

Our study demonstrated that high glucose levels decrease body length and ovulation, and increase the fat storage of *C. elegans*, which may be attributed to the decline in fatty acid and glucose metabolism.

Glucose feeding has a negative effect on the lifespan of *C. elegans* [11-13]. However, few studies have focused on its role in the physical development of worms. One study showed that worms fed on high glucose were larger in size in terms of width [11]. Our work revealed a decrease in *C. elegans* body length. The effect of glucose on ovulation was reported previously [6, 11, 12, 14]. Our study demonstrated a decline in the degree of ovulation in *C. elegans* under high-glucose conditions. Body growth and reproduction of the offspring are energy-intensive processes. However, worms in our study showed a decline in these two physiological functions under high-glucose conditions. Pumping dysfunction and excessive locomotion

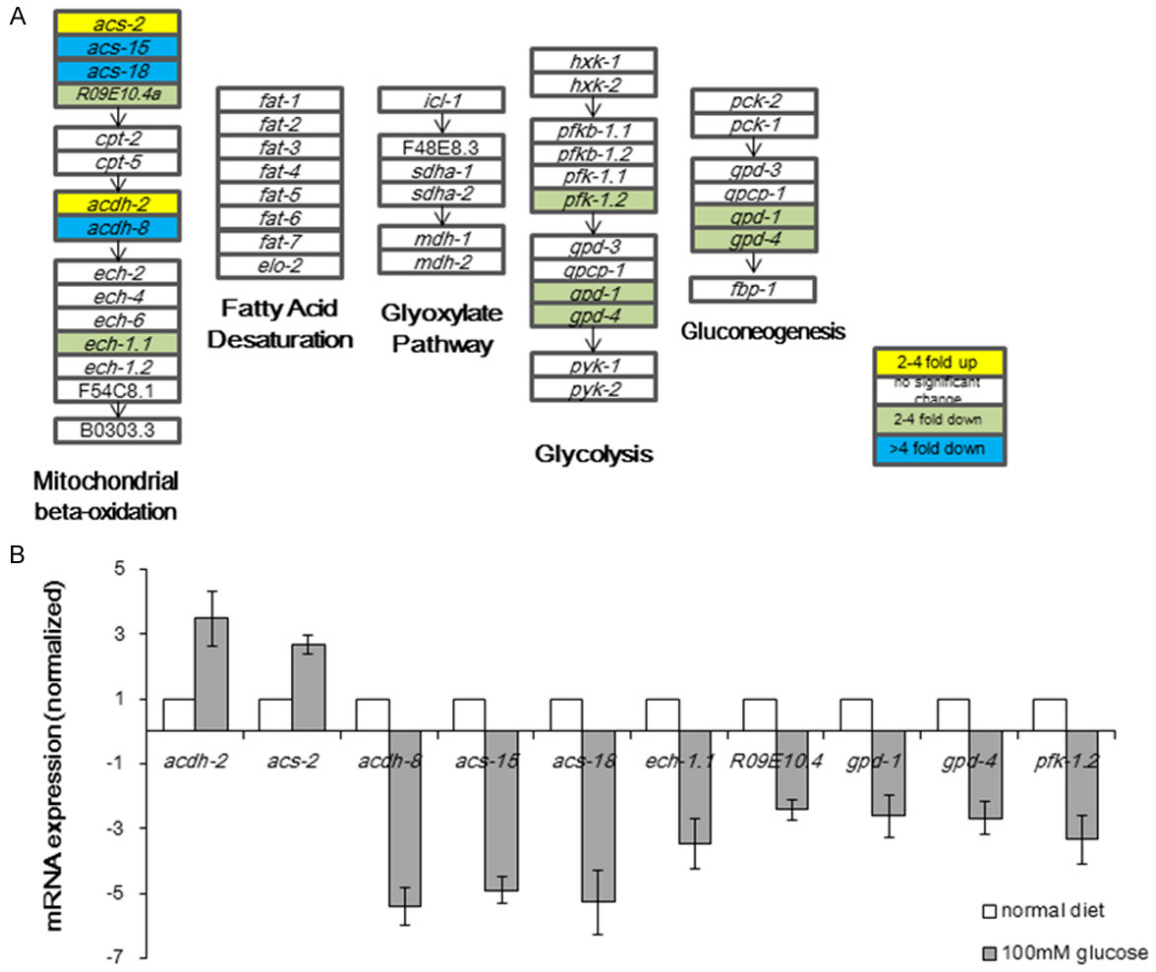

Figure 4. High-glucose diets altered metabolic gene expression. A, B. Ten metabolic genes were significantly altered following exposure to 100 mM glucose. The expression of two genes (*acs-2* and *acdh-2*) was increased whereas eight genes (*acs-15, acs-18, R09E10.4a, acdh-8, ech-1.1, pfk-1.2, gpd-1 and gpd-4*) showed a decline in expression.

were not affected by energy intake or consumption. However, increased fat accumulation was observed.

Therefore, we suspected whether high glucose supply impaired energy utilization. We tested the gene expression of enzymes involved in mitochondrial fatty acid beta-oxidation, fatty acid desaturation and glucose metabolic pathways. We found that a majority of 44 enzymes showed poor expression in fatty acid and glucose metabolism, including three enzymes (*acs-15*, *acs-18*, *acdh-8*) in mitochondrial beta-oxidation, which were dramatically altered. The ACS-15 and ACS-18 belonging to fatty acid CoA synthetase family activate free fatty acids [10]. ACDH-8 is predicted to catalyze acyl-CoA dehydrogenase activity at the start of the mitochondrial fatty acid beta-oxidation [15]. Therefore, the significantly decreased expression of *acs-15*, *acs-18* and *acdh-8* indicates a remarkable decline in fatty acid catabolism.

Glucose restriction induces lipid storage and transport as well as fatty acid oxidation [12]. Our finding confirms the negative relationship between glucose feeding and mitochondrial beta-oxidation. Furthermore, mitochondrial beta-oxidation is a major pathway of fatty acid catabolism contributing to lipid consumption [16]. High glucose influences energy metabolism contrary to the expression of AAK-2, the homolog of AMP-Activated Protein Kinase (AMPK), which may strengthen glucose uptake and utilization as well as fatty acid oxidation [17].

In conclusion, our study found that exposure to high glucose levels negatively affects the physiological function of *C. elegans* via impaired energy utilization. These data provide new insights into the toxicity associated with high-glucose feeding.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Primers for quantitative real-time polymerase chain reaction (qRT-PCR) and 2-ΔΔCt results of *C. elegans* fed with high glucose

Gene	Sequence	Forward/Reverse Primers (5'-3')	2-ΔΔCt
acdh-2	C17C3.12	CGTGTATTCCCACTGCAACA/ATTTCCCGCACAAGAGGTTT	3.47
acdh-8	K05F1.3	TGCTCCGATCTGTTTGTCTG/TCCAGGCTCCGTAACACAAT	0.19
icl-1	C05E4.9	GGTAGTCAAATCGGCTCCAA/TGTGGCAAGGGTGTAGTCAA	0.81
acs-2	F28F8.2	GCCTTGGATGGGATAGAGAG/GGGAAGACCACAGTTTGTCC	2.67
acs-15	R07C3.4	CAATGGCGGAGAGCAATAA/TCAGGTGTTGGTGGAGCAT	0.20
acs-18	R09E10.3	TTACCCGATTTCCGTTCAAG/GCAGCCTTTCTACCTCCATC	0.19
cpt-2	R07H5.2	GCATTTCGTTGCGTTCTACA/GCCGCTTCCTTTCCATTACT	1.56
cpt-5	F09F3.9	GCACCTCCTCAACTGTCGTC/TTTCCGTCCTTCCTCTTTCA	0.88
ech-1.1	C29F3.1	TTGCTTGTCACTACCGCATT/CTTTGAGTTCCACCGTCTCC	0.30
ech-1.2	T08B2.7	CGACACTTCGCACAAACCT/AATCTTCACCACGGCAACAT	1.16
ech-2	F38H4.8	TCCTGTGATGTCGTTGTTGC/TTGGTTGAGCAGTCAGAAGC	1.16
ech-4	R06F6.9	CGGGAATGATGGATTTCGT/CCACCGACCAGTTTAGCATAG	0.83
ech-6	T05G5.6	CGGAGAGAAGGCTCGTTT/TGGTTTCCAGTAAGGCACAC	1.63
elo-2	F11E6.5	AGCACAAACAAGTCCAGCAG/CCGAAGTAGATGGTGACGAGA	1.49
fat-1	Y67H2A.8	TTCTTCGGATTGATGCTCGT/GATGGTTTGGGTTTGTCCAC	1.25
fat-2	W02A2.1	TACACCATCGTCGGTCTTCC/GCAACTCCAGAAACTGCACA	1.42
fat-3	W08D2.4	AAAGCTGCCTAAACAATCTGG/GGAATGGAGGAAGAATGACG	1.26
fat-4	T13F2.1	CGCACATCATCAGTTGTTCA/GCTCTTTCCAACCACCAGAT	0.91
fat-5	W06D12.3	CCTACAAAGCCACCCTCTCA/TCAGCATCAGTATCCGTCCA	1.16
fat-6	VZK822L.1	TGGACTGATACCGATGCTGA/GCTTGGCTCCTTGTTCCTTA	0.92
fat-7	F10D2.9	GCCGTCTTCTCATTTGCTCT/TTGGTGTGGTTGCCTTGTAT	1.49
fbp-1	K07A3.1	TCTGAAGCCTGGAAAGGAAA/GAGTGAACCCATTGACACCA	0.87
gpcp-1	K10B3.6	CTCAAATAACCGATGGATGGA/CGATATTCCTTGTTCGCATAGC	0.53
gpd-1	T09F3.3	ATGGCTTTCCGTGTCCCTAC/CCTTGACTACCTTCTTGATGTCG	0.40
gpd-3	K10B3.7	GACGGACCAATGAAGGGAAT/TGTGGGTTGAGTGAGATGGA	0.95
gpd-4	F33H1.2	AGCTGAACGGAAAGCTCACT/TCCATCGAAGCTGGTTTCTC	0.38
hxk-1	F14B4.2	GAGAGTGTGCCCGAGTTGTT/TTTGTTGGAAAGCAATGAGG	0.84
hxk-2	H25P06.1	CGGTGAGCCGATGAGAACTA/TCGCAGGGATACGAGAAAGT	0.82
mdh-1	F46E10.10	GGAACAACCATCGGAAATGT/CGTAAGCGTCGGTCTCAGTT	0.94
mdh-2	F20H11.3	CTGTCTCCGTCCGTCATTC/CGAGGTGAGCAACAAGTGG	0.77
pck-1	W05G11.6	CTGCGGAAAGACCAACCTC/CTTCGGGATTGATTGCGTA	0.91
pck-2	R11A5.4	CCAAGGATGAAGGATGGATG/CAAGCAGATGGGAAAGCAG	0.98
pfk-1.1	Y71H10A.1	AACAATCACGCCAGAAGAGC/ATCCGACGCAATCTCAGTTC	0.95
pfk-1.2	C50F4.2	AGAAACTGCCAACGAGGAGA/TGGATAGTGGAATGGAAACGA	0.31
pfkb-1.1	Y110A7A.6	TGAGTTCTTCTCGCCAAACA/ATCGCCACTTCTCCTTCCTT	0.82
pfkb-1.2	K02B2.1	GGGACTTCGTGTTTGGTGTT/TCCAGCATCCAGTTCATCG	0.89
pyk-1	F25H5.3	TGATTGTGTGATGCTTTCTGG/AACTGCTGCTTCGGCTTCT	0.75
pyk-2	ZK593.1	TATCGGACCAGCGTGTAGTG/CGGATTGTCTTGATTGTTGC	1.16
sdha-1	C03G5.1	TATTCATCGTTTCCGCTCAA/GTCAAAGCAGTTCCGTCTCC	0.89
sdha-2	C34B2.7	CCGATTCCAGTCATTCCAAC/ATTCACCAGCGGCATAGAGT	0.62
	B0303.3	GAGGTCACGGAGTTGGAATG/AATGGCAGAAATGGGAAGAA	1.32
	F48E8.3	TCTATGTGGTGGGCATTCTG/GGCTTCTGGATTCTCCGATT	0.72
	F54C8.1	ATGGGCAGCAAAGTATCCAG/CGAGTAGAATCCGTCACCAG	0.68
	R09E10.4a	GAAGAGAGTGGCGGAGAAGA/TGTTTCAGGTGTTGGTGGAA	0.42