

## Fructose Induces Transketolase Flux to Promote Pancreatic Cancer Growth

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### Abstract

Carbohydrate metabolism via glycolysis and the tricarboxylic acid cycle is pivotal for cancer growth, and increased refined carbohydrate consumption adversely affects cancer survival. Traditionally, glucose and fructose have been considered as interchangeable monosaccharide substrates that are similarly metabolized, and little attention has been given to sugars other than glucose. However, fructose intake has increased dramatically in recent decades and cellular uptake of glucose and fructose uses distinct transporters. Here, we report that fructose provides an alternative substrate to induce pancreatic cancer cell proliferation. Importantly, fructose and glucose metabolism are quite different; in comparison with glucose, fructose induces thiamine-dependent transketolase flux and is preferentially metabolized via the nonoxidative pentose phosphate pathway to synthesize nucleic acids and increase uric acid production. These findings show that cancer cells can readily metabolize fructose to increase proliferation. They have major significance for cancer patients given dietary refined fructose consumption, and indicate that efforts to reduce refined fructose intake or inhibit fructose-mediated actions may disrupt cancer growth. *Cancer Res*; 70(15); 6368–76. ©2010 AACR.

### Introduction

Cell immortalization and malignant transformation are predominantly determined by altered or aberrant gene expression that in turn modifies cellular metabolic pathways to support tumor growth, viability, and spread. Consequently, cancer cells typically have abnormal metabolism and consume glucose avidly to produce high quantities of lactic acid even in the presence of adequate oxygen, a process first noted by Warburg and colleagues (1). The nonoxidative pentose phosphate pathway (PPP), which allows six-carbon glucose conversion to five-carbon ribose for DNA or RNA synthesis, is of utmost importance for the proliferation process and produces >85% of the ribose recovered from tumor nucleic acids (2). The nonoxidative PPP, controlled by transketolase (TK) enzyme reactions, is encoded by three human TK genes: *TKT*, *TKTL1*, and *TKTL2* (3, 4). Clinically, patients with extensive cancer burden have a tendency to develop thiamine depletion, which is a cofactor for TK-mediated

reactions, emphasizing the importance of the nonoxidative PPP for tumor growth. Increased obesity due to increased total energy consumption and reduced activity now contributes to 15% of U.S. cancer deaths (5, 6). In addition to higher fat intake, a large increase in refined carbohydrate intake has occurred, which itself has been hypothesized to be a risk factor for several cancers (7, 8). Eighty percent of this carbohydrate derives from added sweeteners, in which the disaccharide sucrose (50% glucose and 50% fructose) and high-fructose corn syrup (45% glucose and 55% fructose) are the main sugars (9, 10). Increased refined fructose consumption, in particular, has been highlighted as conferring greater pancreatic cancer risk than other sugars in several recent large epidemiologic studies (11, 12). Normal physiologic glucose concentration, a key substrate for cancer metabolism through glycolysis in the cytosol and through the tricarboxylic acid (TCA) cycle in mitochondria, is tightly controlled between 5 and 7 mmol/L by several hormones, including insulin and glucagon, but little is known about human circulating serum fructose levels (13). We recently observed that mean circulating fasting fructose levels were 2.5 times higher in pancreatic cancer patients in comparison with fasting serum fructose levels in healthy subjects who were in the 0.5 to 1.0 mmol/L range (14). The role, if any, of fructose as a substrate in cancer is poorly understood.

The present study shows that pancreatic cancer cells grow in a range of fructose concentrations that are attainable with the current Western diet and at equivalent rates to glucose. Of major importance, we show that pancreatic cancer metabolism of fructose and glucose is very different, as fructose is a potent inducer of TKT and is preferentially used in the nonoxidative PPP to generate increased nucleic acids in comparison with glucose.

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**Note:** A.P. Heaney conceived and supervised the project and designed the experiments. H. Liu and D. Huang performed the experiments. L.G. Boros designed and performed the metabolomic experiments. D.L. McArthur carried out the biostatistical analysis. N. Nissen provided the surgically resected pancreatic cancer tissues. All authors contributed to the preparation of the manuscript.

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## Materials and Methods

### Cells

Human pancreatic cancer (CaPan-I, CaPan-II, HPAF2, Aspc1, Panc-1, and MiaPaCa-2) and hepatoblastoma (HepG2) cell lines were purchased in 2009 and 2010 from the American Type Culture Collection (ATCC) and used in the described experiments within 6 months after purchase. Authentication testing was performed by ATCC and includes (a) certification that each cell line is negative for *Mycoplasma*, bacteria, and fungi contamination; (b) confirmation of species identity and detection of possible cellular contamination or misidentification using Cytochrome c Oxidase subunit I (COI) for interspecies identification and short tandem repeat (STR) analysis (DNA profiling) for intraspecies identification; and (c) conducting of additional test methods, such as cytogenetic analysis (G-banding and fluorescence *in situ* hybridization), flow cytometry, and immunocytochemistry as well as consistent refinement of cell growth conditions as well as documentation systems, ensuring traceability.

The immortalized pancreatic epithelial cell line HPDE6 was kindly provided by Dr. Stephen Pandol (Veteran's Administration Medical Center, Los Angeles, CA), and all experiments were performed within five passages (15). For primary cultures, freshly resected pancreatic tumors or adjacent normal pancreas tissues were mechanically and enzymatically (trypsin and DNase) disaggregated, and aliquots of tumor cells were seeded in six-well plates. Cell viability was confirmed using a viability kit (Molecular Probes), and keratin staining was used to confirm that >98% of the isolated pancreatic cancer cells were of epithelial origin. Cell aliquots were then incubated in standard DMEM in a range of fetal bovine serum (FBS) concentrations (1–10%) and in a range of glucose or fructose concentrations for 24 to 72 hours.

### *In vitro* proliferation assays

For CellTiter-Glo (CTG; Promega), fluorescence-activated cell sorting (FACS), and bromodeoxyuridine (BrdUrd) proliferation assays,  $1 \times 10^4$  cells were preincubated in standard medium containing 10% FBS (which contains ~0.4 mmol/L glucose) overnight and then plated in a range of glucose- or fructose-containing standard media in 96-well plates for 12 to 96 hours. For CTG assays, proliferation rates were measured according to the manufacturer's instructions. For FACS analysis, treated cells were trypsinized, centrifuged (1,500 rpm  $\times$  2 minutes), washed with PBS, and treated with 20 g/mL RNase A (Calbiochem). DNA was stained with 100  $\mu$ g/mL propidium iodide for 30 minutes at 4°C and protected from light before analysis with a FACScan (Becton Dickinson). For BrdUrd uptake assays, BrdUrd (10  $\mu$ mol/L) was added in the final 12 hours of incubation in the sugars, following which BrdUrd uptake was quantified.

### Metabolomic studies

Confluent cultures (75%) of pancreatic cancer (Panc-1 and MiaPaCa-2), hepatoblastoma (HepG2), and normal pancreatic ductal (HPDE6) cells ( $3 \times 10^6$ ) were incubated for 24 and 72 hours in 5 mmol/L [1,2- $^{13}$ C<sub>2</sub>]D-glucose-containing or

5 mmol/L [1,2- $^{13}$ C<sub>2</sub>]D-fructose-containing (>99% purity and 99% isotope enrichment for each carbon position; Cambridge Isotope Labs) media (half unlabeled glucose/fructose, half labeled with the  $^{13}$ C tracer) in T75 culture flasks. Where possible, these studies were also performed in primary pancreatic tumor and normal pancreas cultures. Briefly, following glucose or fructose treatment, culture medium was collected and cells were washed twice in PBS, after which cell pellets were harvested, specific extractions were performed as described below, and mass spectral data were obtained on a HP5975 inert XL mass selective detector connected to an HP6890N gas chromatograph. A HP-5 capillary column was used for the glucose, ribose, and lactate analyses. Lactate was extracted from cell culture media (0.2 mL) by ethylene chloride after acidification with HCl, derivatized to its propylamine-heptafluorobutyrate ester form, and applied to the column. The  $m/z$  328 (carbons 1–3 of lactate; chemical ionization) were monitored for the detection of  $m_1$  (recycled lactate through the PC) and  $m_2$  (lactate produced by the Embden-Meyerhof-Parnas pathway) for the estimation of pentose cycle activity. For analysis of fatty acid synthesis, palmitate, stearate, cholesterol, and oleate were extracted after saponification of cell pellets in 30% KOH and 100% ethanol using petroleum ether. Fatty acids were then converted to their methylated derivative using 0.5 N methanolic-HCl, and palmitate, stearate, and oleate were monitored at  $m/z$  270,  $m/z$  298, and  $m/z$  264, respectively, with the enrichment of  $^{13}$ C-labeled acetyl units to reflect synthesis, elongation, and desaturation of the new lipid fraction as determined by mass isotopomer distribution analysis (MIDA). To compare ribose and deoxyribose synthesis, RNA ribose was isolated by acid hydrolysis of cellular RNA after Trizol purification of cell extracts. Total RNA was first quantified by spectrophotometric determination in triplicate cultures, and ribose was then derivatized to its aldonitrile acetate form using hydroxylamine in pyridine with acetic anhydride (Supelco) before mass spectral analyses. The ion cluster was monitored around the  $m/z$  256 (carbons 1–5 of ribose; chemical ionization),  $m/z$  217 (carbons 3–5 of ribose), and  $m/z$  242 (carbons 1–4 of ribose; electron impact ionization) to determine molar enrichment and the positional distribution of  $^{13}$ C in ribose. Ribose molecules labeled with a single  $^{13}$ C atom on the first carbon position ( $m_1$ ) recovered from RNA were used to gauge the ribose fraction produced by direct oxidation of glucose or fructose through the glucose-6-phosphate dehydrogenase (G6PDH) pathway. Ribose molecules labeled with  $^{13}$ C on the first two carbon positions ( $m_2$ ) were used to measure the fraction produced by TK. Doubly labeled ribose molecules ( $m_2$  and  $m_4$ ) on the fourth and fifth carbon positions were used to measure molar fraction produced by triose phosphate isomerase and TK.

### TK expression

Quantitative reverse transcription-PCR (RT-PCR) was used to measure TKT, TKTL1, and TKTL2 mRNA levels. Briefly, RNA was extracted from pancreatic cancer cells (CaPan-I, CaPan-II, HPAF2, Aspc1, Panc-1, and MiaPaCa-2) using RNeasy Mini kit (Qiagen). RNA was reverse transcribed

with oligo(dT) primers at 50°C for 50 minutes using the SuperScript III First-Strand Synthesis system (Invitrogen). Real-time PCR was carried out using the SYBR Green qPCR master mix (SABiosciences) in a Bio-Rad MyiQ Single-Color Real-Time PCR Detection System. The primers used for each amplified sequence were designed and purchased from OriGene Technologies, Inc. The cycling parameters for PCR amplification were as follows: activation for 10 minutes at 95°C, then denaturation for 15 seconds at 95°C, and annealing/extension at 60°C for 1 minute (40 cycles). Triplicate  $C_T$  values were analyzed with Microsoft Excel using the comparative  $C_T$  ( $\Delta C_T$ ) method as described by the manufacturer (SABiosciences). The amount of amplified sequence ( $2^{-\Delta\Delta C_T}$ ) was obtained by normalizing to an endogenous reference (18S rRNA) relative to a calibrator (one experimental sample). For immunoblotting, protein from total cell lysates was extracted in radioimmunoprecipitation assay buffer and resolved by the SDS-PAGE by standard methods using antibodies to TK (1:200; Santa Cruz Biotechnology) and  $\beta$ -actin (1:10,000; Santa Cruz Biotechnology).

### TK and uricase activity

Panc-1 pancreatic cancer cells were incubated as before in glucose or fructose for 24 to 120 hours, following which we used an ELISA-based fluorimetric assay (Amplex Red Uric Acid/Uricase Assay kit, Invitrogen) to measure uricase activity in conditioned medium derived from the pancreatic cancer cells. Uricase activity correlates highly with uric acid production, a purine by-product of nucleic acid synthesis. Briefly, in the assay, uricase catalyzes the conversion of uric acid to allantoin, hydrogen peroxide ( $H_2O_2$ ), and carbon dioxide. The  $H_2O_2$  then, in the presence of horseradish peroxidase, reacts stoichiometrically with Amplex Red reagent to generate the red fluorescent oxidation product resorufin, which is measured using excitation at 530 nm and detection at 590 nm. The assay can detect levels as low as 100 nmol/L uric acid. To measure TKT activity, we used a validated method that calculates TKT enzyme activity based on the catalysis of the oxidation of NADH. Briefly, following treatment with glucose or fructose as before, pancreatic cancer cells were lysed in Tris-based protein lysis buffer, sonicated, and spun, and supernatants were collected. One hundred microliters of the latter supernatant fractions were added to a mixture containing 15 mmol/L ribose 5-phosphate, 50  $\mu$ mol/L NADH, 0.1 mmol/L Tris-HCl, and 200 units/mL glycerol-3-phosphate dehydrogenase. After gentle mixing, the absorbance of the mixture was measured at 10-minute intervals for 2 hours at 340 nm. TK activity was then derived from the difference in absorbance at 10 and 80 minutes and expressed as nmol/min/million cells.

### Statistical analysis

Data are presented as mean  $\pm$  SEM except for Fig. 2, where mean  $\pm$  SD and 25% to 75% interquartile ranges are depicted. Student's *t* test (two-tailed) was used to compare two groups, and ANOVA was used for larger group comparisons ( $P < 0.05$  was considered significant).

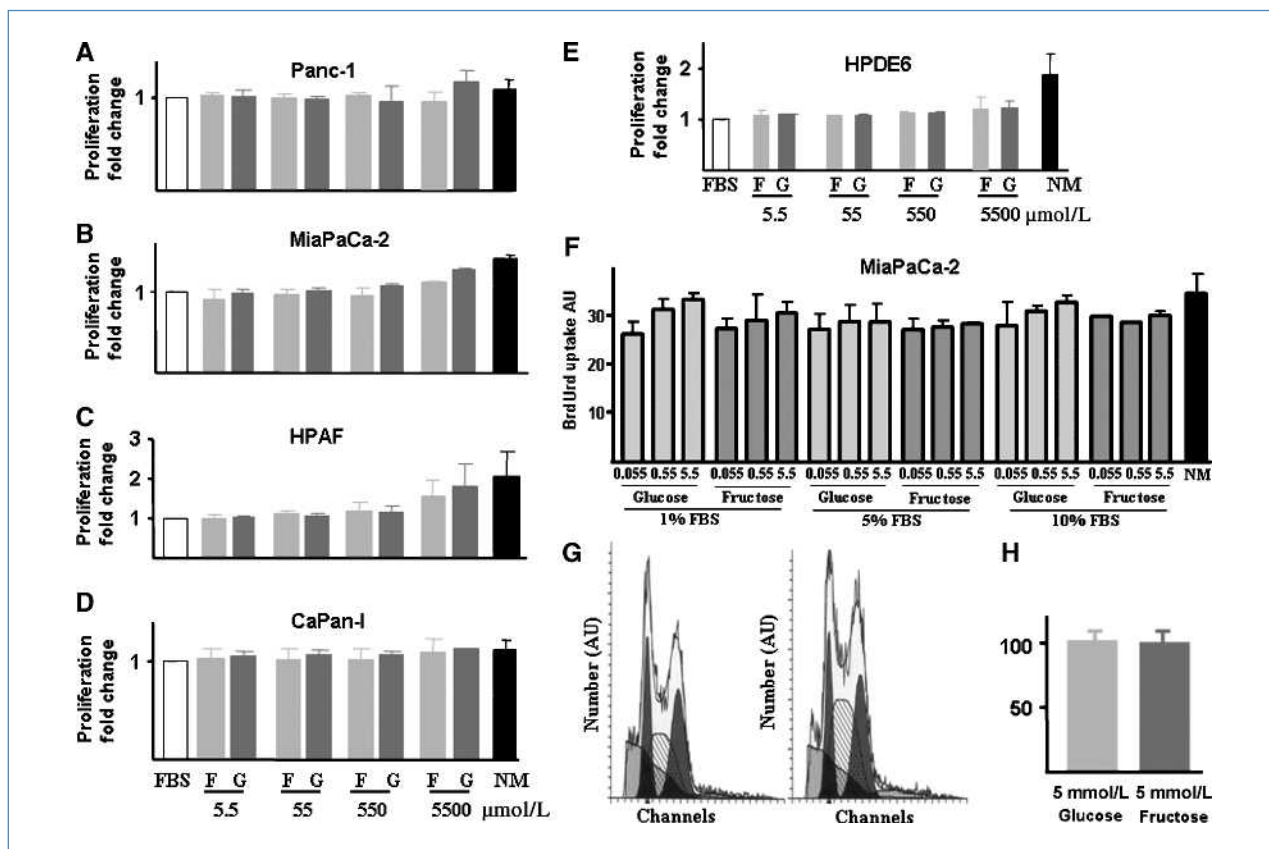
## Results

### Pancreatic cancer cell proliferative rates are similar in fructose and glucose

Typically, pancreatic cancer cells are maintained in high-glucose DMEM that contains  $\sim$ 18 mmol/L glucose, which exceeds normal physiologic (5 mmol/L) or even diabetic (11 mmol/L) glycemic conditions. Little is known about circulating serum fructose levels, and reported concentrations have varied depending on the assay methodology used (16–18). To determine the potential significance of the circulating fructose concentrations we have recently reported in pancreatic cancer patients (14), we measured *in vitro* proliferation rates of several pancreatic cancer cell lines, including Panc-1, MiaPaCa-2, CaPan-I, CaPan-II, and HPAF1, in a range of fructose and glucose concentrations (5–5,500  $\mu$ mol/L) and compared these to cells cultured in 10% FBS alone (which contains 0.4 mmol/L glucose) and standard high-glucose DMEM (normal medium, 18 mmol/L glucose). We also compared glucose- and fructose-induced proliferative rates in immortalized normal pancreatic ductal cells (HPDE6). As expected, the pancreatic cancer cells grew in 55 to 5,500  $\mu$ mol/L glucose (Fig. 1A–D). However, as depicted, the pancreatic cancer cells also grew readily in 55 to 5,500  $\mu$ mol/L fructose (Fig. 1A–D), concentrations that we and others have shown in the human circulation, and proliferative rates were similar in fructose- or glucose-treated cells. The immortalized “normal” pancreatic ductal (HPDE6) cells also grew in both glucose and fructose (Fig. 1E).

As we were examining carbohydrate-mediated effects, we considered that the change we had observed in proliferative rates, as measured by the CTG assay, simply reflected changes in mitochondrial oxidation. Therefore, we used two additional methods to examine proliferative rates following fructose treatment. We also compared BrdUrd uptake in the pancreatic cancer cells following treatment with a range of glucose and fructose concentrations and in a range of FBS concentrations (1%, 5%, and 10%). In agreement with the CTG and FACS studies, similar BrdUrd uptake was observed in glucose-treated in comparison with fructose-treated pancreatic cancer cells (MiaPaCa-2 depicted in Fig. 1F), and the proliferation seemed unaffected by altered FBS levels, showing that fructose serves as an alternate substrate for pancreatic cancer cell proliferation. FACS showed a similar percentage of cells in S phase in Panc-1 pancreatic cancer cells cultured in 5.5 mmol/L glucose in comparison with cells cultured in 5.5 mmol/L fructose, in keeping with equivalent proliferative rates in the two sugars (Fig. 1G; Panc-1: 5.5 mmol/L glucose, 38%; 5.5 mmol/L fructose, 41%;  $P < 0.01$ ). In conjunction with increased S-phase population, a concordant reduction in cells in the resting  $G_0$ - $G_1$  (Panc-1: 5.5 mmol/L glucose, 26%; 5.5 mmol/L fructose, 23%;  $P < 0.05$ ) was noted, supporting the CTG proliferation assay findings and indicating that the pancreatic cancer cells exhibited similar proliferative rates when cultured in equivalent fructose and glucose concentrations.

We also examined proliferative rates in glucose- or fructose-treated primary cultures of freshly resected pancreatic cancers



**Figure 1.** Proliferative effects of glucose and fructose *in vitro*. Proliferative rates of Panc-1 (A), MiaPaCa-2 (B), HPAF (C), and CaPan-1 (D) pancreatic cancer cells and immortalized normal pancreatic ductal HPDE6 cells (E) plated in a range of fructose (F) or glucose (G) concentrations (5  $\mu\text{mol/L}$  to 5 mmol/L) for 48 h measured using a CTG assay. Selected pancreatic cancer cell lines (Panc-1 and MiaPaCa-2) were also plated in 5.5 mmol/L glucose or 5.5 mmol/L fructose for 48 to 72 h, cells were fixed in methanol, and nuclei were stained with propidium iodide for FACS analyses (F), or BrdUrd (10  $\mu\text{mol/L}$ ) was added in the final 12 h of incubation in the sugars, following which BrdUrd uptake was quantified (G). H, mechanically and enzymatically dispersed freshly resected pancreatic cancers were plated in glucose or fructose for 48 h, after which proliferative rates were measured by CTG assay. Proliferative rates (expressed as the percentage difference between the sugars) were measured using a CTG assay. *P* = not significant, fructose versus glucose or normal medium (NM).

( $n = 5$ ). As depicted in a representative cancer (Fig. 1H), proliferation rates were similar in the pancreatic cancer cells treated with either glucose or fructose. These results confirm that although glucose is the preferred substrate for cancer cell proliferation, pancreatic cancer cells can also uptake and use fructose for growth.

#### Cancer cells metabolize fructose and glucose differently

To gain further insight into mechanism(s) by which cancer cells might use fructose versus glucose, leading to cell proliferation, we examined the metabolic profiles of the two sugars in the cancer cells. To do this, we incubated the cells in 5 mmol/L [ $U\text{-}^{13}\text{C}_6$ ]D-glucose or [ $U\text{-}^{13}\text{C}_6$ ]D-fructose (half unlabeled glucose or fructose, half labeled with the  $^{13}\text{C}$  tracer) and analyzed medium and cell pellets by mass spectroscopy to map the metabolic pathways of the two sugars (19, 20). The results derived from the Panc-1 pancreatic cancer cells are presented, and as expected, a large proportion of the  $^{13}\text{C}$ -labeled glucose administered to the pancreatic

cancer cells entered glycolysis and was metabolized to generate lactate (Fig. 2A) and  $\text{CO}_2$  (Fig. 2B). In contrast, a comparatively small fraction of  $^{13}\text{C}$ -labeled fructose was metabolized to generate lactate and  $\text{CO}_2$ , resulting in 800% lower lactate and 350% lower  $\text{CO}_2$  production for fructose in comparison with glucose. Likewise, a significant proportion of glucose metabolism contributed to fatty acid synthesis as evidenced by glucose-derived  $^{13}\text{C}$ -labeled behenic acid (Fig. 2C, light bar) and palmitate (Fig. 2D, light bar), but 150% lower levels of behenic acid (Fig. 2C, dark bar) and palmitate levels (Fig. 2D, dark bar) were derived from  $^{13}\text{C}$ -labeled fructose (similar results were seen for all  $\text{C}_{16}$  to  $\text{C}_{26}$  fatty acids).

#### Fructose is preferentially used for nucleic acid synthesis

The pentose phosphate shunt comprises an oxidative branch regulated by the enzyme G6PDH and a nonoxidative branch regulated by TKT (21). Metabolomic studies showed that both sugars were similarly used via the G6PDH-regulated oxidative pathway of the PPP (Fig. 2E). However, in contrast

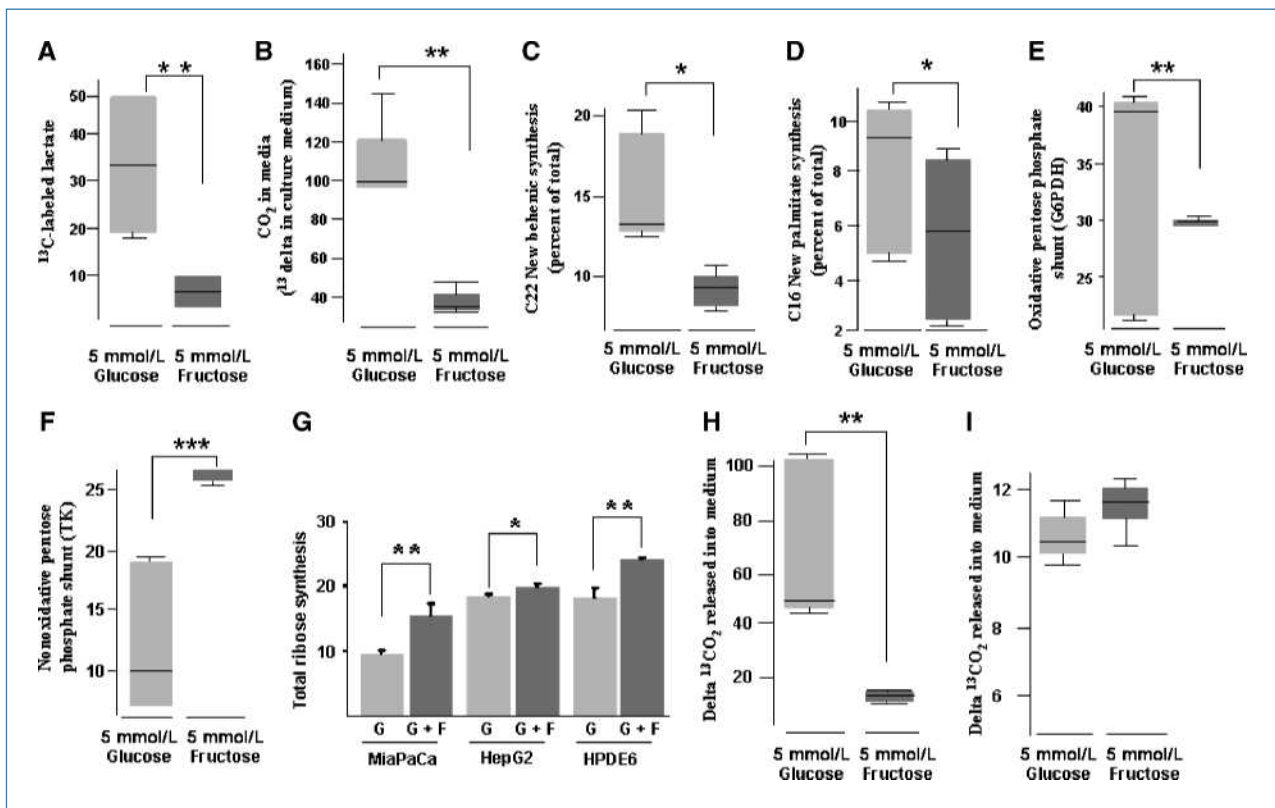


to the relatively low contribution of fructose to glycolysis and fatty acid synthesis in comparison with glucose, metabolomic studies showed that  $^{13}\text{C}$ -labeled fructose was preferentially metabolized at 250% higher rates than glucose via the TK-regulated, nonoxidative pathway of the PPP to synthesize nucleic acids (Fig. 2F). In support of the effects observed in pancreatic cancer Panc-1 cells, addition of fructose (5 mmol/L) to other solid cancers including pancreatic cancer MiaPaCa, hepatoblastoma HepG2 cells, and immortalized normal pancreatic ductal cells (HPDE6) incubated in glucose (5.5 mmol/L) resulted in increased ribose synthesis via the TK-regulated nonoxidative pathway (Fig. 2G). We also carried out metabolomic studies in freshly resected pancreatic carcinoma cultures using the  $[\text{U-}^{13}\text{C}_6]\text{D}$ -glucose or  $[\text{U-}^{13}\text{C}_6]\text{D}$ -fructose tracers. Results derived from the freshly resected cancers concurred with the findings in the pancreatic cancer cell lines and showed that glucose was oxidized in pancreatic cancers at 460% higher rates in comparison with fructose (Fig. 2H). However, in normal pancreas tissues, oxidative use of glucose and fructose to generate  $\text{CO}_2$  was

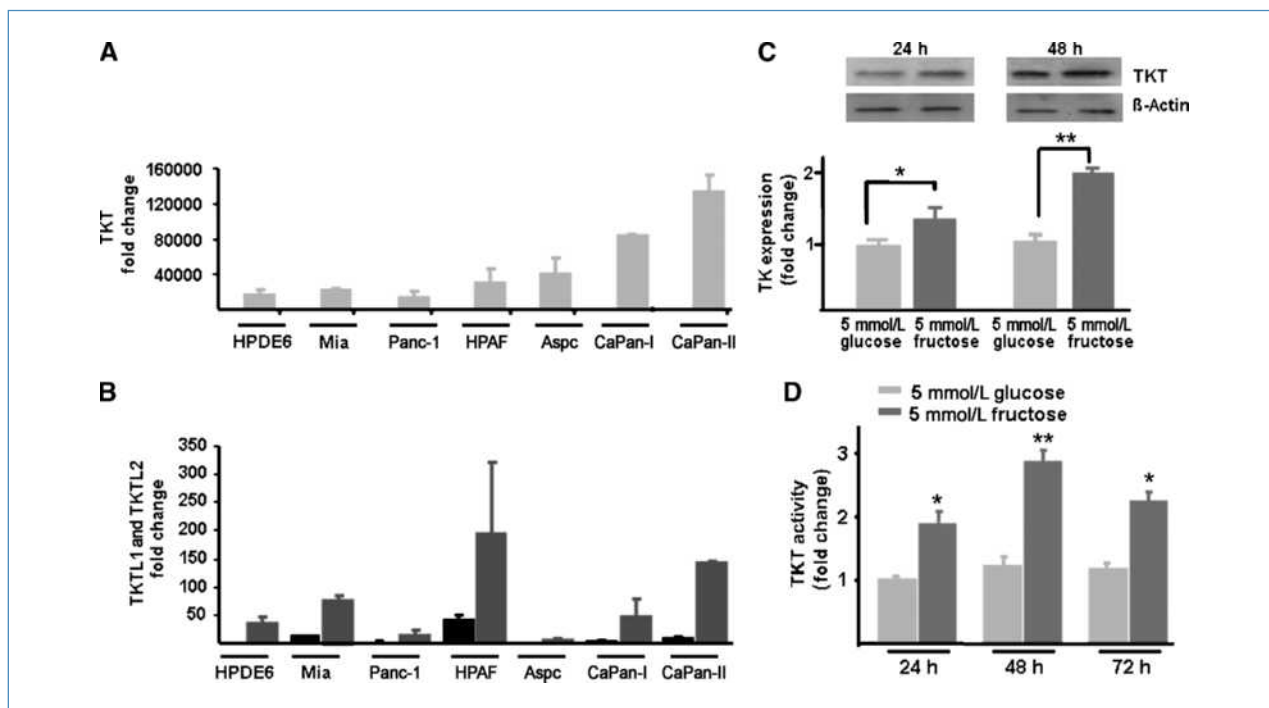
similar (Fig. 2I), suggesting that differential use of fructose versus glucose is restricted to transformed pancreatic cells.

### Fructose induces TK-dependent purine synthesis

The enzyme TKT drives nucleic acid synthesis by generation of xylulose-5-phosphate from fructose-6-phosphate and glycerol (21). Recent studies have shown increased TKT gene expression in several cancers, and TKT levels correlate with reduced survival rates (3, 4). As depicted (Fig. 3A and B), all the pancreatic cancer cell lines tested expressed TKT, TKTL1, and TKTL2, although TKT mRNA (Fig. 3A) levels were significantly ( $\sim 300$ - to  $800$ -fold) higher than TKTL1 and TKTL2 (Fig. 3B). Additionally, as depicted in Fig. 3A, a trend toward higher baseline TKT mRNA levels was seen in moderately and well-differentiated pancreatic cancer cell lines, although low-level TKT was also seen in normal pancreatic ductal HPDE6 cells. To explore the potential mechanism by which fructose might be preferentially used in the nonoxidative pathway in comparison with glucose, we then asked if fructose treatment could modify TKT expression



**Figure 2.** Metabolomic studies in fructose- and glucose-treated pancreatic (Panc-1 and MiaPaCa-2) and liver (HepG2) cancer cells and normal pancreatic ductal (HPDE6) cells. A, mass spectroscopic analysis of label incorporation into lactate, the main three-carbon product of glycolysis, following incubation of 75% confluent cultures of Panc-1 cells ( $3 \times 10^6$ ) for 72 h in 5 mmol/L  $[\text{1,2-}^{13}\text{C}_2]\text{D}$ -glucose-containing or 5 mmol/L  $[\text{1,2-}^{13}\text{C}_2]\text{D}$ -fructose-containing media (half unlabeled glucose/fructose, half labeled with the  $^{13}\text{C}$  tracer). B, hexose oxidation in the pentose and TCA cycles to generate  $\text{CO}_2$  production. C and D, enrichment of  $^{13}\text{C}$ -labeled acetyl units reflecting synthesis, elongation, and desaturation of new lipid fractions as determined by MIDA of behenic acid (C) and palmitate (D). E and F, rates of  $^{13}\text{C}$  incorporation from glucose or fructose in pancreatic cancer Panc-1 cells through the oxidative (E) and nonoxidative (F and G) branches of the PPP into RNA ribose or DNA deoxyribose and through the nonoxidative PPP in pancreatic [Panc-1 (F) and MiaPaCa-2] and liver (HepG2) cancer cells and normal pancreatic ductal (HPDE6) cells (G). H and I, oxidative use of glucose and fructose in representative primary cultures of a pancreatic ductal cancer (H) and normal pancreas tissue (I). \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

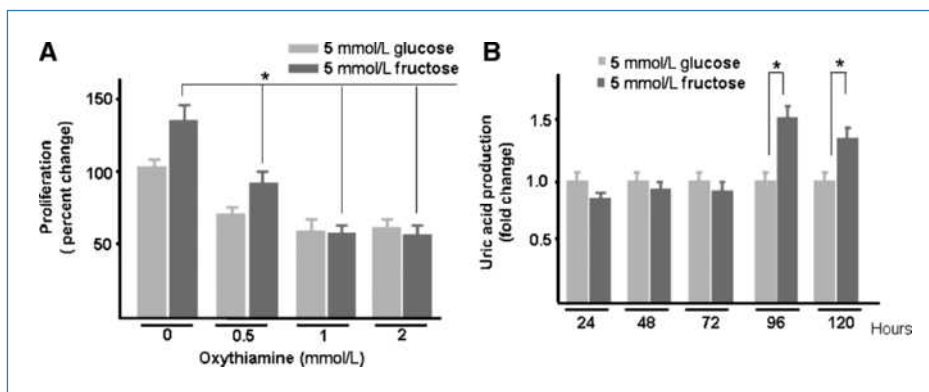


**Figure 3.** Fructose induces pancreatic cancer TK. Quantitative RT-PCR of TKT (A) and TKTL1 and TKTL2 (B) mRNA expression in immortalized normal pancreatic ductal HPDE6 cells and MiaPaCa-2, Panc-1, HPAF, Aspc1, CaPan-I, and CaPan-II pancreatic cancer cells. C, Western blot analysis of TK protein expression in glucose- and fructose-treated pancreatic cancer Panc-1 cells for 24 and 48 h. TKT levels were normalized to  $\beta$ -actin as depicted in the bottom panel. D, TKT enzyme activity following glucose and fructose treatment (24, 48, and 72 h) was determined based on the catalysis of the oxidation of NADH.

and/or action. Following Panc-1 cell fructose treatment, TKT protein expression increased  $200 \pm 3\%$  in comparison with glucose-treated cells (Fig. 3C). Moreover, in addition to increased TKT levels, TKT activity increased  $280 \pm 5\%$  following fructose treatment (Fig. 3D) in comparison with TKT activity in the glucose-treated Panc-1 cells. The enzyme TKT requires thiamine-derived mitochondrial  $NAD^+$  as a cofactor, and  $NAD$  synthesis can be disrupted with the thiamine analogue oxythiamine. In keeping with our finding that fructose-induced pancreatic cancer proliferation involved TKT, oxythiamine treatment abolished the fructose-induced increased pancreatic cancer cell proliferation rates (Fig. 4A). We reasoned that if fructose was preferentially used for nucleic acid synthesis, that levels of uric acid, the purine synthesis waste by-product, might also alter following fructose treatment. Consistent with the metabolomic studies that showed increased fructose-derived nucleic acid synthesis, uricase activity in conditioned medium harvested at 96 and 120 hours from the fructose-treated Panc-1 cells was  $\sim 120\%$  and  $\sim 150\%$  higher than levels measured in conditioned medium derived from the glucose-treated Panc-1 cells at the same time points ( $P < 0.01$ ; Fig. 4B). These results showed increased pancreatic cancer cell uric acid production after fructose treatment, and as uric acid is a by-product of purine metabolism, this observation further supported our metabolomic studies that showed that fructose was metabolized to a greater extent than glucose to generate nucleic acids.

## Discussion

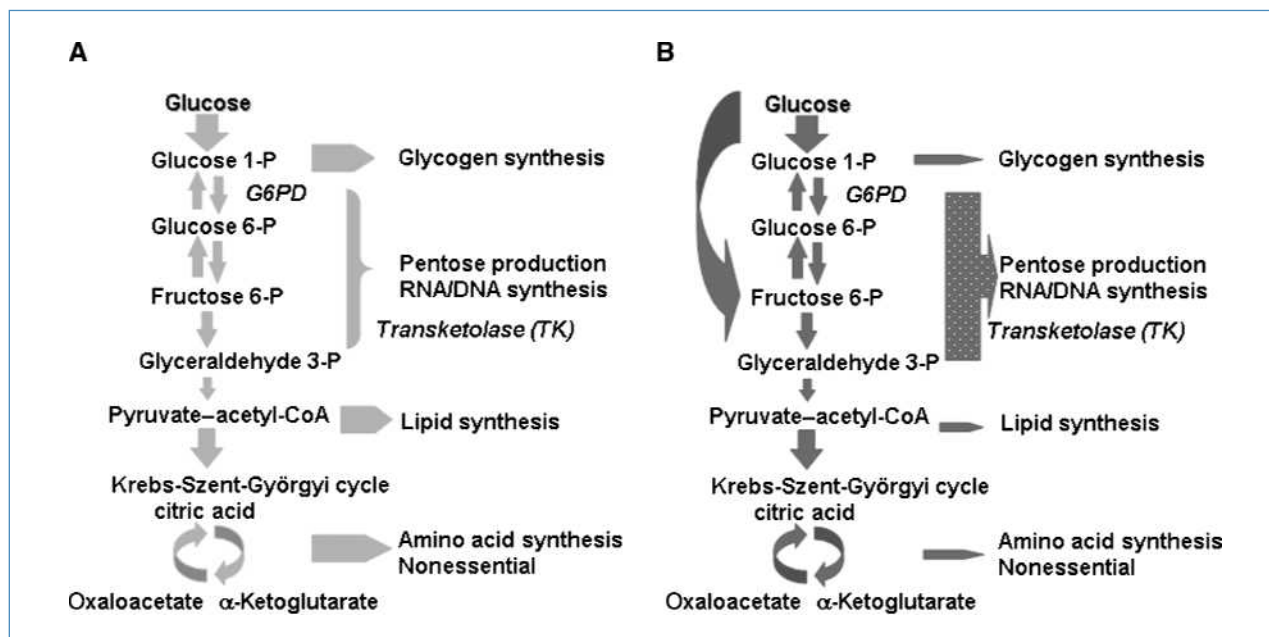
Conventionally, fructose and glucose have been considered as interchangeable monosaccharides that are both metabolized equivalently in aerobic glycolysis and in the TCA cycle, contributing equally to fatty acid and nucleic acid synthesis as required by cell demands (22, 23). However, our data show that metabolism of fructose and glucose by cancer cells is importantly different in ways that do not simply involve fructose-glucose isomerization. Indeed, our data indicate that the contribution of fructose to nucleic acid synthesis is considerably greater than glucose and that cancer cells preferentially use fructose via TKT-mediated metabolism to synthesize additional nucleic acids to facilitate increased proliferative capacity. Synthesis of nucleic acids and nucleotides is of utmost importance for proliferating tissues and especially cancers. Central reactions of this process include ribose 5-phosphate formation through phosphoribosyl  $PP_i$ , and prior studies in pancreatic cancer cells have shown that pentose cycle reactions contribute to  $>85\%$  of *de novo* ribose synthesis in RNA with the majority derived from the non-oxidative (TKT-regulated) pathway (21). This is the first report of TKT gene expression in pancreatic cancer cells and shows expression of TKT, TKTL1, and TKTL2 in all the pancreatic cancer cell lines tested. TKT mRNA levels were significantly higher in all pancreatic cancer cell lines, and a trend toward higher baseline TKT mRNA levels was seen in moderately and well-differentiated pancreatic cancer cell lines.



**Figure 4.** Inhibition of fructose-induced pancreatic cancer Panc-1 cell proliferation by (A) incubation with the TK inhibitor oxythiamine (0.5–2 mmol/L) for 72 h. Proliferative rates were measured using the MTS proliferation assay. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ . B, uric acid production was measured after glucose or fructose treatment by uricase-mediated catalytic conversion of uric acid to allantoin, carbon dioxide, and  $H_2O_2$ , which in the presence of horseradish peroxidase reacts stoichiometrically with Amplex Red reagent to generate the red fluorescent oxidation product resorufin.

However, low-level TKT was also seen in normal pancreatic ductal HPDE6 cells, and therefore, the significance of this heterogeneous TKT mRNA expression is uncertain. Studies in other tumor types, including cervical, colorectal, thyroid, glioma, and renal cancers, showed that tumor TKTL1 mRNA was overexpressed in comparison with normal tissues, and TKTL1 mRNA levels correlated with reduced survival rates (4). Furthermore, experimental suppression of TKTL1 action by small interfering RNA (siRNA), short hairpin RNA (shRNA), or specific TK inhibitors reduced cancer cell proliferation, and activation of TK by thiamine addition stimulated tumor growth (2, 3). Our results are similar in that higher TKTL1 levels were seen in pancreatic cancers than

in normal pancreatic ductal cells. However, our results clearly illustrate that TKT is the predominantly expressed gene encoding TK in pancreatic cancer and normal pancreatic ductal cells. Suppression of TK action by siRNA or shRNA or by pharmacologic inhibition results in reduced cancer cell proliferative rates due to reduced glucose consumption, lower lactate production, and ultimately impaired supply of ATP for cancer cell growth. In this study, we show that fructose potently induces TKT expression and activity to aid metabolism of fructose in the PPP. Although the PPP is inefficient at ATP generation, it functions to provide C5 sugars (pentoses) from C6 sugars (hexoses), simultaneously generating NADPH redox equivalents. These pentoses are urgently needed for



**Figure 5.** Schematic model summarizing the differences in glucose (A) and fructose (B) metabolism in pancreatic cancer cells showing the preferential use of fructose in the TK-dependent nonoxidative pentose phosphate shunt.

DNA and RNA synthesis and allow access to NADPH redox equivalents, which may be essential to counter endogenous (from dysfunctional mitochondria) and exogenous (from immune cells) reactive oxygen species that are increased in cancers. The role of fructose to generate nucleic acids is supported by our demonstration of increased production of uric acid, as a by-product of purine metabolism, and provides important mechanistic insight into a recent report of increased serum uric acid levels in patients with high-fructose consumption (24). Thus, we propose an alternative model for glucose (Fig. 5A) and fructose (Fig. 5B) metabolism in cancer cells, where fructose is preferentially used to generate nucleic acids in comparison with its contribution to glycolysis and fatty acid synthesis. In addition to our *in vitro* findings in cancer cells, previous studies have shown that chronic fructose feeding in animals leads to insulin resistance and promotes *in vivo* growth, as evidenced by increased organ weights (25, 26). Additionally, in humans, increased fructose consumption has been linked to obesity, diabetes, and elevated uric acid levels, the latter in keeping with our *in vitro* metabolomic studies showing increased fructose-directed nucleic acid synthesis (26, 27). We have recently shown 2.5-fold higher serum fructose levels in pancreatic cancer patients compared with healthy subjects (14). Furthermore, in healthy volunteers, serum fructose level rose rapidly following ingestion of a liquid fructose and glucose load, and in contrast to glucose that quickly returned to fasting levels, serum fructose remained elevated for >2 hours, suggesting that circulating human fructose levels are unregulated in comparison with the exquisite regulation of blood glucose (14).

In summary, fructose, either in the hepatic portal or systemic human circulation, provides cancer cells a plentiful direct source of donor keto-groups for the pentose-synthesizing TK enzyme (2, 13). Our results are extremely important for pancreatic cancer growth for two reasons (28). First, they show that fructose itself is preferentially used via the nonoxidative PPP to provide five-carbon pentose for RNA synthesis. Second,

fructose induces TK expression, an activity that enables more rapid use of both glucose and fructose in the nonoxidative PPP. We established this using the highest precision scoring metabolic tracer [1,2-<sup>13</sup>C<sub>2</sub>]D-glucose as recently reported from the Massachusetts Institute of Technology (29). Together, these fructose-mediated actions enable pancreatic cancers to more rapidly generate increased nucleic acids and proliferate more efficiently. The findings should also heighten awareness about the independent contribution of other sugars, in this case fructose, to cancer metabolism and the cancer phenotype.

Therefore, fructose is a particularly significant dietary sugar component with important implications for patients with cancer, particularly given the significant dietary change that has occurred in human fructose consumption since the mid-20th century. Our findings provide important insights into recent epidemiologic studies that have identified refined fructose as an independent risk factor for pancreatic cancer, and identify fructose-mediated actions as a novel therapeutic cancer target.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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#### References

- Warburg O, Posener K, Negelein E. On the metabolism of cancer cells. *Biochem Z* 1924;152:319–44.
- Boros LG, Puigjaner J, Cascante M, et al. Oxythiamine and dehydroepiandrosterone inhibit the nonoxidative synthesis of ribose and tumor cell proliferation. *Cancer Res* 1997;57:4242–8.
- Xu X, Hausen AZ, Coy JF, Lochelt M. Transketolase-like protein 1 (TKTL1) is required for rapid cell growth and full viability of human tumor cells. In *J cancer* 2009;124:1330–7.
- Staiger WI, Coy JF, Grobholz R, et al. Expression of the mutated transketolase TKTL1, a molecular marker in gastric cancer. *Oncol Rep* 2006;16:657–61.
- National Institutes of Health: National Institute of Diabetes, Digestive and Kidney Diseases. "Statistics related to overweight and obesity: the economic costs." Available from: <http://www.win.niddk.nih.gov/statistics/index.htm>.
- Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among U.S. children, adolescents, and adults, 1999–2002. *JAMA* 2004;291:2847–50.
- Calle EE, Thun MJ. Obesity and cancer. *Oncogene* 2004;23:6365–78.
- Kaaks R, Lukanova A. Effects of weight control and physical activity in cancer prevention. International agency for research on cancer. *Ann N Y Acad Sci* 2002;963:268–81.
- Park YK, Yetley EA. Intakes and food sources of fructose in the United States. *Am J Clin Nutr* 1993;58:737–47S.
- Bray GA, Nielson SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am J Clin Nutr* 2004;79:537–43.
- Michaud DS, Liu S, Giovannucci E, et al. Dietary sugar, glycemic load, and pancreatic cancer risk in a prospective study. *J Natl Cancer Inst* 2002;94:1293–300.
- Larsson SC, Bergkvist L, Wolk A. Consumption of sugar and sugar-sweetened foods and the risk of pancreatic cancer in a prospective study. *Am J Clin Nutr* 2006;84:1171–6.
- Kim JW, Dang CV. Cancer's molecule sweet tooth and the Warburg effect. *Cancer Res* 2006;66:8927–30.
- Hui H, McArthur D, Nissen N, Heaney AP. Direct spectrophotometric determination of serum fructose in pancreatic cancer patients. *Pancreas* 2009;38:706–12.
- Ouyang H, Mou LJ, Luk C, et al. Immortal human pancreatic duct epithelial cell lines with near normal genotype and phenotype. *Am J Pathol* 2000;157:1623–31.



16. Shiota M, Galassetti P, Monohan M, et al. Small amounts of fructose markedly augment net hepatic glucose uptake in the conscious dog. *Diabetes* 1998;47:867–73.
17. Sucha R, Ulcova-Galova Z, Pavelkova-Seifertova P, et al. Fructose and glucose in follicular fluid and serum of women undergoing stimulation in an *in vitro* fertilization program. *Ceska Gynekol* 2002; 67:144–8.
18. Richthoff J, Spano M, Giwercman YL, et al. The impact of testicular and accessory sex gland function on sperm chromatin integrity as assessed by the sperm chromatin structure assay (SCSA). *Hum Reprod* 2002;17:3162–9.
19. Cascante M, Boros LG, Comin B, Atauri P, Centelles JJ, Lee PW. Metabolic control analysis in drug discovery and disease. *Nat Biotechnol* 2002;20:246–9.
20. Boros LG, Cascante M, Lee WN. Metabolic profiling of cell growth and death in cancer: applications in drug discovery. *Drug Discov Today* 2002;7:364–72.
21. Lee WN, Boros LG, Puigjaner J, Bassilian S, Lim S, Cascante M. Mass isotopomer study of transketolase-transaldolase pathways of the pentose cycle with [1,2-<sup>13</sup>C<sub>2</sub>]glucose. *Am J Physiol* 1998;274: E843–51.
22. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis. *Nat Rev Cancer* 2004;4:891–9.
23. Eigenbrodt E. Glycolysis: one of the keys to cancer? *Trends Pharmacol Sci* 1980;1:240–5.
24. Nakagawa T, Tuttle KR, Short RA, Johnson RJ. Hypothesis: fructose-induced hyperuricemia as a causal mechanism for the epidemic of the metabolic syndrome. *Nat Clin Pract Nephrol* 2005; 1:80–6.
25. Tobey TA, Mondon CE, Zavaroni I, Reaven GM. Mechanism of insulin resistance in fructose-fed rats. *Metabolism* 1982;31:608–12.
26. Koh ET, Mueller J, Osilesi O, Knehans A, Reiser S. Effects of fructose feeding on lipid parameter in lean, diabetic and nondiabetic Zucker rats. *J Nutr* 1985;115:1274–84.
27. Putnam JJ, Allshouse JE. Food consumption, prices, and expenditures, 1970-97. Washington (DC): Economic Research Service, U.S. Department of Agriculture; 1999.
28. Bardeesy N, DePinho RA. Pancreatic cancer biology and genetics. *Nat Rev Cancer* 2002;2:897–909.
29. Metallo CM, Walther JL, Stephanopoulos G. Evaluation of <sup>13</sup>C isotopic tracers for metabolic flux analysis in mammalian cells. *J Biotechnol* 2009;144:167–74.

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