Metabolic Responses to Fructose-1,6-Diphosphate in Healthy Subjects

Angel K. Markov, William A. Neely†, Ralph H. Didlake, Joe Terry III, Alan Causey, and Patrick H. Lehan

Fructose-1,6-diphosphate (FDP) is an important naturally occurring intracellular metabolite with a direct regulatory role in many metabolic pathways. The most important and widely studied of the FDP effects has been its regulation of glycolysis, particularly the enzyme that synthesizes FDP—phosphofructokinase (PFK). Since it was observed experimentally that FDP does indeed modulate carbohydrate metabolism, we investigated whether FDP would similarly enhance carbohydrate utilization in man. The study used indirect calorimetry and was open to healthy adults (N = 45) of either sex and above legal age. After a steady metabolic state was obtained, 5 g of FDP (10%) was infused into a brachial vein. In 10 subjects, glucose (5 g) or FDP (5 g) was sequentially infused. The rapid intravenous infusion of FDP produced a slight but significant decrease in heart and respiration rates ($P < .05$). A significant increase in the serum concentration of inorganic phosphate ($P < .0001$) and the intraerythrocytic concentration of adenosine triphosphate (ATP) ($P < .01$) was also observed. The FDP infusion produced a decrease in plasma cholesterol and triglycerides ($P < .001$ and $P < .01$, respectively). The indirect calorimetric data indicate that the infusion produced a highly significant increase in the respiratory quotient ([RQ] $P < .0001$) and the energy derived from carbohydrates ($P < .0001$) and a significant decrease in the energy derived from lipids ($P < .0001$). Glucose infusion did not cause changes in any of the parameters. These data indicate that carbohydrate metabolism is stimulated by FDP.

ALL OF THE INTERMEDIATES in glycolysis are esters of phosphoric acid, but only the initial substrate glucose and the final products pyruvic acid and lactic acid are not phosphorylated compounds. The phosphate group is most important in glycolysis because it is a chemical vehicle necessary for resynthesizing adenosine triphosphate (ATP) from adenosine diphosphate. However, there are other important biochemical biological properties of these phosphorylated compounds. Most notable among these is the intermediate fructose-1,6-diphosphate (FDP). FDP is a naturally occurring intracellular metabolite that is intimately linked to the regulation of many metabolic pathways.[1] It augments carbohydrate utilization by stimulating glycolysis while simultaneously inhibiting gluconeogenesis. FDP prevents glycogen breakdown and stimulates its synthesis.[1]

However, the most intensive investigations into the primary effect of FDP have focused on its regulatory effect on glycolysis and particularly the effect on phosphofructokinase (PFK), the enzyme that synthesizes FDP.[1-5] These observations indicate that FDP is a potent activator of PFK.[1,3-5] Additionally, in glycolysis, FDP acts as an activator of liver pyruvate kinase and isoforms in other tissues and organs excluding skeletal muscle.[1,6,7]

Considerable attention has focused on PFK activity in hypoxic and ischemic conditions.[8-11] In such circumstances, studies have shown that PFK is inhibited by acidosis, thereby inactivating the Embden-Meyerhoff pathway, an alternative source of energy production in ischemia and hypoxia.[8-11] Theoretically, one approach to alleviate this metabolic block would be to provide exogenous FDP, since FDP has the capacity to stimulate PFK activity directly and, at the same time, to be used anaerobically as a high-energy substrate. The use of FDP to enhance energy production from the Embden-Meyerhoff pathway in ischemic hypoxic and hypoperfusion states has been addressed in a number of studies.[12-23]

In experimental and clinical myocardial infarction, FDP administration improves hemodynamics, attenuates electrocardiographically assessed injury and arrhythmia, attenuates ATP and creatine phosphate depletion from ischemic myocardium, reduces infarct size, and increases survival.[12,13,21,22] FDP treatment of animals subjected to hemorrhagic shock results in significantly reduced mortality rates.[20] The success rate of cardiopulmonary resuscitation with FDP after hypoxic cardiac arrest is significantly increased.[16] FDP has been shown to have salutary effects on renal, intestinal, and brain ischemia.[15,16,18,19] In patients with compromised heart function secondary to coronary artery disease (CAD), short-term treatment with FDP significantly improves left-ventricular performance.[24]

The theoretical rationale for using FDP in the treatment of ischemia and shock is thus strong. However, to substantiate that this agent indeed has an effect on metabolism as described, we were prompted to investigate whether FDP is capable of producing the same metabolic effect in humans as in animal models. This task was accomplished with the aid of indirect calorimetry, and the clinical and laboratory findings obtained before, during, and after rapid intravenous infusion of FDP compared with glucose infusion are reported.

SUBJECTS AND METHODS

The purpose of this study was to substantiate that FDP stimulates carbohydrate metabolism in human subjects as has been documented in animal models. The investigation was performed in compliance with local regulations and the directives outlined by the Institutional Review Board. Each subject was fully informed of the medication, the study aim, and the clinical procedure. Consent forms were signed by each subject and by witnesses.

Subjects

The study was open to healthy adults of either sex and above legal age. Exclusion criteria were as follows: history of chronic disease, fructose intolerance, allergic reactions, hypersensitivity to intravenous administration, pregnancy, concomitant medications, and failure to sign

From the Division of Cardiovascular Diseases, Department of Medicine, and Department of Surgery, University of Mississippi School of Medicine, Jackson, MS.

†Deceased.

Submitted August 24, 1998; accepted December 2, 1999.

Address reprint requests to Angel K. Markov, MD, Division of Cardiovascular Diseases, Department of Medicine, University of Mississippi School of Medicine, 2500 N State St, Jackson, MS 39216.

0026-0495/00/4906-0011$10.00/0

doi:10.1053/meta.2000.6249

consent forms. Forty-five subjects (mean age, 32.1 ± 9.5 years) participated in the study, of whom 35 were male and 10 female (age range: male, 24 to 58; female, 23 to 61), with a mean body weight of 78.9 ± 12.8 kg (range, 59 to 114).

Drug Preparation and Administration

FDP (Esafosfina) was supplied by Biomedica Foscama (Rome, Italy) in vials for intravenous infusion. Each vial contained 5 g FDP sodium salt lyophilized powder. The content was reconstituted with 50 mL pyrogen-free distilled water to yield a 10% solution of FDP and then administered via the antecubital vein as an intravenous infusion over a period of 9.6 ± 1.6 minutes (Due to the small caliber of the catheterized vein in 3 females, 14, 16, and 17 minutes were required to complete the infusion.) To verify whether glucose administration would affect metabolism in the same manner, 10 subjects first received either FDP or dextrose (injection USP in water obtained from the hospital pharmacy) 5 g (10%) in a crossover manner, after a baseline metabolic steady state was achieved. At the end of the study, the subjects were asked to collect a urine sample for determination of the renal excretion of FDP.

Experimental Procedures and Assessments

One or 2 days prior to the calorimetric studies and FDP infusion, each subject was instructed to collect the total overnight urine output (noting the time) and bring it to the laboratory for hourly urinary nitrogen determinations. The urine samples were analyzed by a Coleman (Wichita, KS) nitrogen analyzer.

On the study day, a general physical examination and laboratory tests were conducted. The subject reclined on a bed, and a butterfly (19-gauge) catheter was inserted into a brachial vein, with patency ensured by a slow intravenous drip of 0.9% NaCl (Travenol, Deerfield, IL). Vital signs were monitored and continuous electrocardiographic monitoring with intermittent recordings was started. A transparent canopy was placed about the head of the subject, metabolic data acquisition began, and a steady metabolic state was normally obtained between 15 and 25 minutes, ie, there were no changes in calorimetric values for the last 10 1-minute determinations. At that time, FDP was infused while calorimetric data were collected and printed at 1-minute intervals. After the FDP or glucose infusions were terminated, the collection of metabolic data continued for an additional 25 to 35 minutes.

The laboratory studies included all hematological (complete blood cell count) and blood chemistry (SMA-18) screens routinely used for patients admitted for observation. Posttreatment blood samples were obtained at 10 and 25 minutes after infusion for repeat laboratory analysis.

Indirect Calorimetry Equipment and Calibrations

The indirect calorimetric system consists of 3 basic units: instrumentation, computer, and program.[25] Air is drawn from the canopy where the head of the individual is placed. An in-line dry-test meter measures the air flow by sending electrical impulses to the AD converter of the computer. A gas sample from the flow-measuring instrument is pumped to a Drierite column (W.A. Hammond Drierite, Xenia, OH) to remove all water vapor, and then divided through 2 rotameters to a Beckman (Fullerton, CA) paramagnetic oxygen analyzer and infrared carbon dioxide analyzer. The output signal of these instruments is amplified, converted from analog to digital form, and fed into a computer. The program processes the oxygen concentration, carbon dioxide concentration, and flow as a readout (corrected for barometric pressure and temperature) of oxygen consumption, carbon dioxide production, a respiratory quotient (RQ), grams of carbohydrate, fat, protein, and energy from each foodstuff, and total energy produced in watts per meter squared. Protein metabolism is computed from the subject's hourly urinary nitrogen level, which is entered into the computer. The Boyd equations are used by the computer to solve for these parameters.[26]

Prior to each study, the system was calibrated with standardized gas mixtures in varying proportions of oxygen and carbon dioxide. After termination of the study, an alcohol lamp was weighed, placed under the canopy, and burned for approximately 15 minutes. The lamp was removed and reweighed, and the amount of alcohol burned was recorded. The volume of oxygen consumed (in liters) and carbon dioxide produced (in liters) and the weight of alcohol burned (in grams) were entered into the computer, and the percent error in O_2 consumption and CO_2 production was determined.

Data Processing and Analysis

Data were analyzed with a personal computer using standard software (Microsoft Excel Version 7.0). All data are presented as the mean ± SE. The paired 2-tailed Student's *t* test was used for statistical analysis of the fluctuation of the parameters from the basal values during and after FDP or glucose infusion. Differences in mean values for preinfusion, during infusion, and after FDP or glucose infusion were detected by paired or unpaired 2-tailed Student's *t* test and ANOVA as appropriate. The relationship of changes in energy production (watts per meter squared) from carbohydrates and lipids induced by glucose and FDP was analyzed with linear regression analysis. *P* values less than .05 were considered significant.

Analyses of the calorimetric data were performed in the Department of Surgery using a Gimix 6809 (Motorola, Chicago, IL) computer. The original printout of the calorimetric data became an integral part of each subject's file. Blood chemistry and hematology analyses were performed in the University Hospital laboratories. Other biochemical data such as ATP, FDP (blood and urine), dihydroxyacetone phosphate (DHAP), pyruvate, and lactate were analyzed in the Department of Medicine Cardiovascular Research laboratories.[27-29]

RESULTS

The infusion of 5 g FDP did not affect systolic and diastolic blood pressure. Similarly, no electrocardiographic changes were noted. However, the heart rate decreased from 69.6 ± 12.4 to 64.7 ± 10.7 bpm ($P < .05$) and the respiration rate from 11.3 ± 1.3 to 10.9 ± 1.1 breaths/min ($P < .05$). There was a slight but significant ($P < .025$) increase in plasma sodium following FDP (from 141.6 ± 0.54 to 142.6 ± 0.52 mEq/L), as one should expect because FDP is a trisodium salt. A similar slight increase was observed in blood CO_2 (from 22.3 ± 0.52 to 22.9 ± 0.52 mEq/L, $P < .05$). Plasma potassium and chloride values did not change significantly. Plasma inorganic phosphate increased substantially following FDP administration (3.55 ± 0.132 to 6.06 ± 0.116 mg/dL, $P < .0001$). Cholesterol and triglycerides after FDP infusion decreased from 186 ± 6.9 to 171 ± 6.5 mg/dL ($P < .001$) and from 61.8 ± 6.9 to 54 ± 6.5 mg/dL ($P < .01$), respectively. Blood glucose concentrations were not different before (86.6 ± 2.8 mg/dL) and after (86.1 ± 2.5 mg/dL) FDP infusion. The FDP blood concentration increased from 1.13 ± 0.1 to 3.39 ± 0.7 mg/dL ($P < .001$) postinfusion, whereas pyruvate and lactate declined from 1.22 ± 0.08 to 0.956 ± 0.05 mg/dL ($P < .005$) and from 7.66 ± 0.61 to 6.34 ± 0.55 mg/dL ($P < .005$), respectively. However, the lactate to pyruvate ratio remained unchanged. It should be noted that blood DHAP, the first product of FDP metabolism, increased from 0.0529 ± 0.04 μmol/mL to 0.0785 μmol/mL ($P < .005$). ATP from whole homogenized blood increased from 2.97 ± 0.13 to 3.19 ± 0.16 μmol/mL ($P < .005$) following FDP administration.

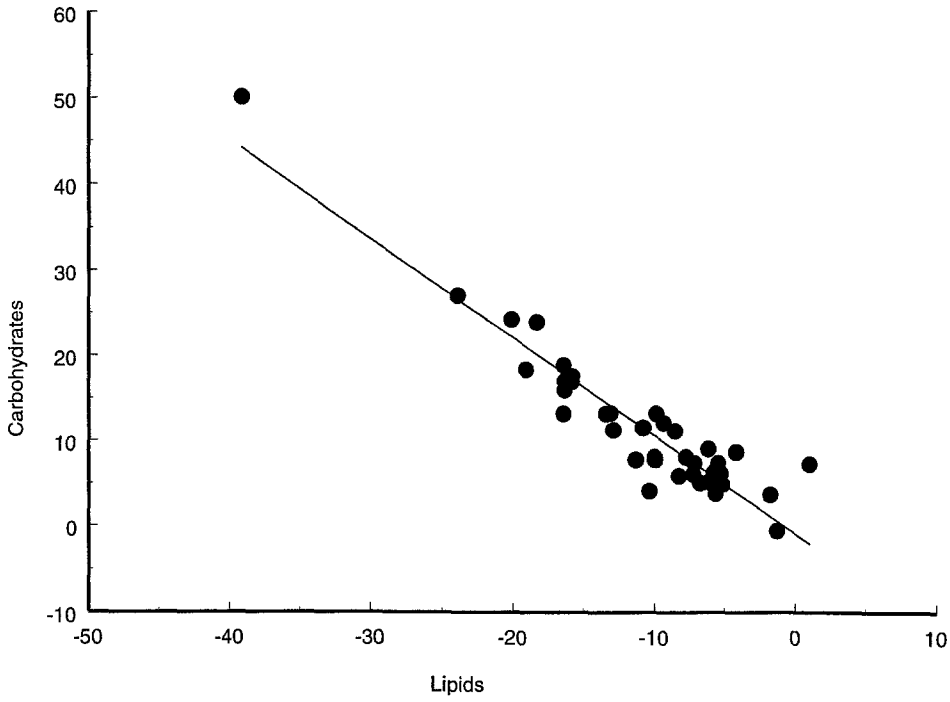

Fig 1. Relationship between changes in carbohydrate and lipid energy production (W/m^2) induced by FDP administration demonstrating a strong correlation: −.9411, $R^2 = .886$, $P = 5.2 \times 10^{-18}$.

During the infusion, FDP induced a significant increase in carbohydrate utilization ($P < .0001$), and this effect persisted, although to a lesser degree, until the end of the study period ($P < .05$). While carbohydrate utilization was increased in a similar manner, there was a parallel decline in lipid utilization ($P < .0001$). These changes in carbohydrate-lipid utilization induced by FDP were strongly correlated ($R^2 = .886$; Fig 1). A summary of the calorimetric data is presented in Tables 1 and 2. It should be noted that infusion of only 5 g FDP produced a highly significant augmentation in the RQ, from 0.833 to 0.900 ($P < .0001$).

Calorimetric data obtained in the 10 subjects who received 5 g FDP or 5 g glucose are presented in Table 3. Again, FDP produced an effect on carbohydrate-lipid energy production similar to that shown in Fig 1. These changes demonstrated good correlation (−.740, $R^2 = .548$, $P = .014$). Glucose infusion produced no changes in carbohydrate-lipid energy production ($R^2 = .255$, P = NS). None of the previously mentioned vital signs or blood chemistry parameters were affected by the infusion of dextrose, except for a significant increase in blood glucose from 84 ± 3.67 to 102 ± 3.01 mg/dL ($P < .001$). FDP produced the same effect on the parameters as previously indicated. Only 3% to 4% of infused FDP was found as such in the urine.

DISCUSSION

FDP infusion produced an increase in the RQ, while glucose failed to augment carbohydrate utilization. After termination of the infusion, this effect of FDP persisted for another 15 to 20 minutes. It appears that this increase in carbohydrate utilization is probably linked to the ability of FDP to intervene in glycolysis as a metabolic regulator.[1-5] In normoxic conditions, the activity of PFK is inhibited by ATP and citrate. However, data from in vitro experiments suggest that a significantly high concentration of FDP might modulate the activation process of PFK.[1-5] It is of interest to note that FDP also acts as an activator of liver pyruvate kinase.[6,7] The results support the consideration that FDP may have, in part, enhanced the carbohydrate metabolism by such a mechanism. As a consequence of increased carbohydrate utilization, there was a proportional decrease in lipid oxidation, with no change in total energy production (watts per meter squared). Hence, despite an increased utilization of one substrate in the basal state, the organism preserves energy homeostasis (Table 2).

To interpret our results correctly, it is necessary to consider certain principles on which indirect calorimetry is based. First, oxygen diffuses rapidly at the cellular level, and the average transit time between alveolar air and mitochondria is about 2

Table 1. Effect of FDP on O_2 Consumption (mL/min), CO_2 Production (mL/min), and RQ in Healthy Subjects During and After Infusion

	O_2 Consumption			CO_2 Production			RQ		
	Basal	During	After	Basal	During	After	Basal	During	After
Mean	263.4	258.7*	260.1	219.7	240†	222	0.833	0.900‡	0.85†
SEM	5.5	5.1	5.2	5.1	8.2	4.4	0.016	0.012	0.011

NOTE. Only the RQ remained elevated during the postinfusion period *v* basal ($P < .01$).

*$P < .05$.

†$P < .01$.

‡$P < .0001$.

Table 2. Energy Production: Total and for Each Foodstuff (W/m²)

	Carbohydrates			Lipids			Total		
	Basal	During	After	Basal	During	After	Basal	During	After
Mean	16.6	26.9*	19.2†	19.5	11.3*	16.6‡	44.6	44.6	44.2
SEM	1.72	1.9	1.32	2.1	1.4	1.53	0.71	0.71	0.69

NOTE. Since the hourly urinary nitrogen was determined prior to FDP administration, protein metabolism was considered to remain unchanged during the study period. The contribution from this source to total energy production before, during, and after FDP infusion was 8.5 ± 0.39 W/m².

*$P > .0001$, basal *v* during.

†$P > .05$, ‡$P > .001$: basal *v* post.

minutes. Under the conditions of the present study, the oxygen body pool is very small (≈1.2 L) and variations in oxidative metabolism are translated into equivalent variations in expired oxygen. Thus, an error from this source, if any, should be negligible. However, carbon dioxide is distributed in a very large body pool (~16 L, excluding bone) compared with the global daily flux of 300 L under resting conditions.[30] Therefore, a delay prior to changes in the expired carbon dioxide is expected and may depend on the individual bicarbonate kinetics.[31] Because the study was performed in the resting state and for 10 minutes prior to FDP infusion, values for the expired carbon dioxide remained unchanged at each 1-minute interval, and it is reasonable to assume that a steady state was achieved. Variations in the pH and bicarbonate pool during FDP infusion seem unlikely, because with infusion of twice as much FDP (150 mg/kg) in patients with CAD over the same period, no changes in arterial and venous pH were noted.[24] The least likely source of an error is the protein metabolism. Ferrannini[30] suggests that a large error in the measurement of urinary nitrogen will produce an error of about 1% to 2% in true energy production. The participants in the present study had no history of chronic disease, their renal function was normal, and urinary nitrogen measurements and urine collections were performed according to clinical standards. Thus, in the present study, the observed changes in substrate utilization are believed to reflect the ability of FDP to enhance glycolysis.

Since the plasma glucose concentration did not change following FDP infusion, one could stipulate that the agent partly metabolized as such. Several parameters indicate that this might be the case. First, there was an increase in DHAP ($P < .005$) in the blood, which is the first by-product of FDP metabolism. Second, considering that 98% of total red blood cell energy production is derived from anaerobic glycolysis, the significant increase in intraerythrocytic ATP indicates that FDP may have been partially used as a substrate in addition to stimulating glycolytic activity. Other investigators have observed a similar intraerythrocytic increase in high-energy adenyl nucleotides when incubated with only FDP.[32] Thus, it appears that FDP was used, at least in part, as substrate. That only 3% to 4% of the infused FDP was found as such in the urine also indicates that a portion of it may have been metabolized. These considerations are in agreement with reports in the literature indicating that FDP can be used as a substrate.[18,33,34] Blood lactate and pyruvate actually decreased ($P < .005$) despite increased carbohydrate utilization via the oxidative process. However, the lactate to pyruvate ratio remained the same as observed prior to FDP infusion. In this connection, total energy production remained unchanged despite the 39% increase in energy production derived from carbohydrates. Thus, the decrease in lactate and pyruvate, with no change in their ratio, could be attributed to curtailed lipid utilization (Table 2). Because glucose and lipid metabolism are related, energy production depends on which substrate is the preferential producer.[35] Another argument in favor of this explanation is that glucose infusion did not produce any changes in the carbohydrate and lipid utilization or lactate and pyruvate concentration (data not shown). Since glucose was used as the control, one may argue that phosphorylated hexoses or inorganic phosphate might have produced effects similar to FDP. As reported, the action of FDP appears to be specific and not shared by other phosphorylated sugars such as glucose-6-phosphate and fructose-1-phosphate.[36-38] Neither fructose nor glucose alone or in combination with inorganic phosphate has been shown to have a salutary effect.[20,36-39] As stated in the Introduction, the rationale for the use of FDP in ischemic and hypoperfusion states is that this agent intervenes in the Embden-Meyerhoff pathway as both metabolic regulator and high-energy substrate.[1-23] The salutary effect of FDP in ischemia/hypoxia and shock has been attributed to its ability to enhance energy production via glycolysis. The cited reports tend to support this contention.[12-24]

FDP is reported to afford ischemic/hypoxic protection to different organs, including the heart, kidney, intestine, and brain. It must be pointed out that with indirect calorimetry, it is not possible to differentiate which tissues and organs contrib-

Table 3. Effects of FDP or Glucose Administration in 10 Subjects on Total Energy Production and Energy Production From Each Foodstuff

	Carbohydrates (W/m²)			Lipids (W/m²)			RQ			Total Energy (W/m²)		
	CON	GLUC	FDP	CON	GLUC	FDP	CON	GLUC	FDP	CON	GLUC	FDP
Mean	11.5	10.5	18.6*	25.69	26.3	18.02†‖	0.804	0.799	0.857‡¶	44.6	43.7	44.2
SEM	2.38	1.86	2.04	3.56	2.82	2.64	0.018	0.016	0.015	1.18	1.34	1.04

NOTE. Glucose infusion produced no changes in the RQ or carbohydrate and lipid utilization (NS). In this group, FDP produced the same effects as noted in Table 2. The contribution of protein metabolism to total energy production was 8.4 ± 0.34 W/m².

Control (CON) *v* infusion: *$P < .005$, †$P < .002$, ‡$P < .001$.

FDP *v* glucose (GLUC): §$P < .005$, ‖$P < .05$, ¶$P < .005$.

uted most to the enhanced energy production from carbohydrate metabolism.[12,13,15,16] The contribution of various organs and tissues to basal metabolism depends on their respective oxygen consumption and proportion of body weight. Skeletal muscle constitutes about 40% of body weight, but it consumes 2.3 mL/min/kg; thus, it contributes approximately 25% to basal metabolism. On the other hand, the contribution of major organs to basal metabolism is about 60% (liver 26%, brain 18%, heart 9%, and kidney 7%). Therefore, it is most likely that the observed increase in carbohydrate utilization reflects the predominant contribution of skeletal muscle, because FDP is a much stronger activator of muscle PFK than of the liver isoenzyme. The results of this study indicate that FDP is capable of increasing carbohydrate utilization even in normoxic conditions. In conjunction with its metabolic effect in ischemia/hypoxia, these findings support that, by such a mechanism, FDP exerts the reported therapeutic effects.[12-20]

The significant decrease in plasma cholesterol and triglycerides induced by FDP is difficult to explain, because the calorimetric data clearly show that lipid utilization was curtailed. An examination of the calorimetric data for individual subjects reveals that FDP always decreased lipid metabolism regardless of the preinfusion RQ (range, 0.69 to 0.98). On the other hand, glucose produced no changes in lipid metabolism or plasma lipid concentrations. The significance of the phenomenon cannot be assessed at this time. However, it is reported that long-term (3 to 25 days) FDP administration to Watanabe heritable hyperlipidemic rabbits significantly decreased plasma cholesterol and triglycerides (by 27% and 50%, respectively).[40] In this connection, it is suggested that changes in hepatic lipid metabolism induced by FDP may account for these observations.

The issue of whether FDP enters the cells remains controversial because of the assumption that phosphorylated compounds do not cross cellular membranes. Investigations have demonstrated that radiolabeled FDP crosses myocardial cellular membranes and can be used as a substrate at normoxia.[33] Gregory et al[36] observed a rapid incorporation of [^{14}C]-FDP into the cell fraction of normoxic astrocytes at 37°C. However, they noted that [^{14}C]-FDP uptake at 0°C decreased by 70%, suggesting an energy-dependent process. Cyanide-poisoned brain tissue can maintain adequate ATP levels if FDP is added to the media, and if incubations are performed in glucose-free media.[18] The most convincing evidence that FDP, to some extent, can gain access to the cytosol is reported by Hardin and Roberts.[34] Using a nuclear magnetic resonance technique, these investigators showed that ^{13}C-FDP enters smooth muscle cells and is metabolized during normoxia and hypoxia to ^{13}C-lactate.[34]

In conclusion, the acute metabolic effects of FDP in healthy humans were investigated, with the following observations: FDP increased the RQ by preferentially enhancing carbohydrate utilization, and lipid metabolism was diminished while total energy production remained constant. Extrapolating from these results, it appears that exogenous FDP can modulate the activity of PFK and be used in part as a substrate.

REFERENCES

1. Kirtley ME, McKay M: Fructose-1,6-diphosphate, a regulator of metabolism. Mol Cell Biochem 18:141-149, 1977
2. Passoneau JV, Lowry OH: Phosphofructokinase and the Pasteur effect. Biochem Biophys Res Commun 7:10-16, 1962
3. El-Badry M, Ontoni A, Mansour TE: Studies on heart phosphofructokinase. Role of fructose diphosphate in enzymatic activity. J Biol Chem 248:557-563, 1973
4. Mansour TE: Studies on heart phosphofructokinase; purification, inhibition, and activation. J Biol Chem 238:2285-2292, 1963
5. Hood K, Holloway R: The significant role of fructose-1,6-diphosphate in regulatory kinetics of phosphofructokinase. FEBS Lett 68:8-14, 1976
6. Taylor CB, Bailey E: Activation of liver pyruvate kinase by fructose 1,6-diphosphate. Biochem J 102:32-33, 1967
7. Iurovitskii IUG, Milman LS: Regulation of pyruvate kinase activity by fructose-1,6-diphosphate in developing embryo. Dokl Akad Nauk SSSR 175:1404, 1967
8. Wollenberger A, Krause EG: Metabolic control characteristics of acutely ischemic myocardium. Am J Cardiol 22:349-359, 1968
9. Kubler W, Spieckermann PG: Regulation of glycolysis in the ischemic and anoxic myocardium. J Mol Cell Cardiol 1:351-377, 1970
10. Opie LH, Owen P: Glycolysis in acute experimental myocardial infarction: Pathways of metabolism and preliminary results, in Bayusz E, Rona G (eds): Recent Advances in Studies on Cardiac Structure and Metabolism, vol 2. Baltimore, MD, University Park, 1973, pp 567-578
11. Rovetto MJ, Lamberton WF, Neely JR: Mechanisms of glycolytic inhibition in ischemic rat hearts. Circ Res 37:742-751, 1975
12. Markov AK, Oglethorpe NC, Blake TM, et al: Hemodynamic, electrocardiographic, and metabolic effects of fructose diphosphate on acute myocardial ischemia. Am Heart J 100:639-646, 1980
13. Zhan JN, Zhange FM, Ma WS, et al: Protective effect of exogenous fructose-1,6-diphosphate in cardiogenic shock. Cardiovasc Res 22:927-932, 1988
14. Webb WR: Metabolic effects of fructose diphosphate in hypoxic and ischemic states. J Thorac Cardiovasc Surg 88:863-866, 1984
15. Didlake RH, Kirchner K, Lewin J, et al: Attenuation of ischemic renal injury with fructose-1,6-diphosphate. J Surg Res 47:220-226, 1989
16. Sun J, Farias LA, Markov AK: Fructose 1-6 diphosphate prevents intestinal ischemic reperfusion injury and death in rats. Gastroenterology 98:117-126, 1990
17. Farias LA, Willis MM, Gregory GA: The effects of fructose diphosphate, glucose and saline on cardiac resuscitation. Anesthesiology 65:595-601, 1986
18. Bickler PE, Kelleher JA: Fructose 1,6-bisphosphate stabilizes brain intracellular calcium during hypoxia in rats. Stroke 23:1617-1622, 1992
19. Farias LA, Smith EE, Markov AK: Prevention of ischemic-hypoxic brain injury and death in rabbits with fructose 1-6 diphosphate. Stroke 21:606-613, 1990
20. Markov AK, Terry JW, White RH, et al: Increasing survival of dogs subjected to hemorrhagic shock with fructose 1,6-diphosphate. Surgery 102:515-527, 1987
21. Marchionni N, Conti A, De Alfieri W, et al: Hemodynamic and electrocardiographic effects of fructose 1,6-diphosphate in acute myocardial infarction. Am J Cardiol 56:266-269, 1985
22. Fu YJ, Zhang DW, Gu JY, et al: Fructose 1,6-diphosphate reduces mortality and improves haemodynamics in patients with acute myocardial infarction. Eur Heart J 16:442, 1995 (suppl, abstr)
23. Markov AK: Hemodynamics and metabolic effects of fructose 1-6 diphosphate in ischemia and shock—Experimental and clinical observations. Ann Emerg Med 15:1470-1477, 1986
24. Markov AK, Brumley MA, Figueroa A, et al: Hemodynamic effects of fructose-1,6-diphosphate in patients with normal and impaired left ventricular function. Am Heart J 133:541-549, 1997

25. Neely WA, Petro AB, Holloman GH, et al: Research on the cause of burn hypermetabolism. Ann Surg 179:291-294, 1974

26. Boyd WC: Error in calibration of commercial BMR machines. J Appl Physiol 6:711, 1954

27. Lamprecht W, Trautschold I: Adenosine-5′-triphosphate: Determination wth hexokinase and glucose-6-phosphate dehydrogenase, in Bergmeyer HU (ed): Methods of Enzymatic Analysis. New York, NY, Academic, 1974, pp 2101-2110

28. Marbach EP, Weil MH: Rapid enzymatic measurement of blood lactate and pyruvate. Clin Chem 13:314-325, 1967

29. Bucher T, Hohorst JH: Dihydroxyacetone phosphate, fructose-1,6-diphosphate and D-glyceraldehyde 3-phosphate, in Bergmeyer HV (ed): Methods of Enzymatic Analysis. New York, NY, Academic, 1963, pp 246-252

30. Ferrannini E: The theoretical basis of indirect calorimetry. Metabolism 37:287, 1988

31. Simonson DC, DeFronzo RA: Indirect calorimetry: Methodological and interpretative problems. Am J Physiol 258:E399, 1990

32. Mangalini SI, Bondoli A, Scracia E: The action of phosphocreatine and fructose-1,6-diphosphate on blood in vitro. Resuscitation 5:102-110, 1976

33. Tavazzi B, Starnes JW, Lazzarino G, et al: Exogenous fructose-1,6-bisphosphate is a metabolizable substrate for the isolated normoxic rat heart. Basic Res Cardiol 87:280-289, 1992

34. Hardin CD, Roberts TM: Metabolism of exogenously applied fructose 1,6-bisphosphate in hypoxic vascular smooth muscle. Am J Physiol 267:H2325-H2332, 1994

35. Randle PG, Garland PB, Hales CN, et al: The glucose fatty acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1:785-789, 1963

36. Gregory GA, Yu Albert CH, Chan Pak H: Fructose-1,6-bisphosphate protects astrocytes from hypoxic damage. J Cereb Blood Flow Metab 9:29-34, 1989

37. Oyanagui Y: Fructose-1,6 diphosphate enhanced oxyradicals and nitric oxide–dependent suppression by dexamethasone of ischemic and histamine paw edema of mice. Life Sci 62:241-249, 1998

38. Planas ME, Sánchez S, González P, et al: Protective effect of fructose 1,6-bisphosphate against carrageenan-induced inflammation. Eur J Pharmacol 237:251-255, 1993

39. Iapichino G, Scherini A, Maritano M, et al: 2,3-Diphosphoglycerate (DPG) blood levels in polytransfused patients: Effects of inorganic and organic phosphate infusion. IRCS Med Sci 8:231-232, 1980

40. Markov AK, Didlake RH, Bigelow C, et al: Lowering of plasma lipids with fructose-1,6-diphosphate in animal model and man. J Mol Cell Cardiol 26:83, 1994 (abstr)