Route dependent effect of nutritional support on liver glucose uptake

Sheng-Song Chen
Carlos J Torres-Sanchez
Nadeen Hosein
Yiqun Zhang
D. Brooks Lacy
Chris Chang
Owen P. McGuinness

Department of Molecular Physiology and Biophysics
Vanderbilt Univ. School of Medicine
Nashville, TN 37232-0615
USA

Running Title: Route dependency of nutritional support

Key words: intestine, glycogen

Address all reprints to:
Owen P. McGuinness, Ph.D.
702 Light Hall
Dept. Molecular Physiology and Biophysics
Vanderbilt University
Nashville, TN 37232-0615
Phone 615-343-4473
FAX 615-322-1462
E-mail: owen.mcguinness@vanderbilt.edu
Abstract:
The liver is a major site of glucose disposal during chronic (5 days) total parenteral (TPN) and enteral (TEN) nutrition. Net hepatic glucose uptake (NHGU) is dependent upon the route of delivery when only glucose is delivered acutely, however the hepatic response to chronic TPN and TEN is very similar. The aims were to determine if the route of nutrient delivery altered the acute (first 8 h) response of the liver and if chronic enteral delivery of glucose alone could augment the adaptive response to TPN.

Chronically catheterized conscious dogs received either TPN or TEN containing glucose, Intralipid and Travasol either for 8 h or 5 days. Another group received TPN for 5 days but ~50% of the glucose in the nutrition was given via the enteral route (TPN+EG).

Hepatic metabolism was assessed using tracer and arterio-venous difference techniques. In the presence of similar arterial plasma glucose levels (~6 mM) NHGU and net hepatic lactate release increased ~2-fold between 8h and 5 days in TPN and TEN. NHGU (26±1 vs.23±3 µmol/kg/min) and net hepatic lactate release (44±1 vs. 34±6 µmol/kg/min) in TPN+EG were similar to TPN, despite lower insulin levels (96±6 vs. 58±16 pM; TPN vs. TPN+EG). TEN does not acutely enhance NHGU or disposition above that seen with TPN. However partial delivery of enteral glucose is effective in decreasing the insulin requirement during chronic TPN.
**Introduction**

In stressed states (trauma, injury, or infection) nutritional support is often provided to patients via either the parenteral (TPN) or enteral (TEN) route. Prior studies suggest that when the nutrition (either TPN or TEN) is given continuously for 5 days liver glucose uptake is markedly augmented; the liver removes approximately 45% of the exogenous glucose (1). Even more surprisingly the substantial in liver glucose uptake occurred in the absence of hyperglycemia (~6.7 mmol/L) and only mild hyperinsulinemia (102 pmol/L) (1). Recently we observed that the enhancement in the capacity of the liver to take up glucose (i.e. adaptive response) begins within 5 h after initiation of TPN and is nearly fully manifest by 24h(3).

The enteral route is the preferred route for delivery of exogenous nutrients (12, 14). In post surgical and stressed patients isocaloric enteral nutrition can be given without significant accompanying hyperglycemia(22, 23) as compared to TPN. A potential benefit of the enteral route is that when only glucose is delivered via the enteral route in the acute setting it enhances NHGU to a greater extent than when glucose is delivered via a peripheral route; this route dependent effect has been termed the "portal signal" (7, 20). The “portal signal” can rapidly (<15min) augment NHGU; it does not require the presence of hyperglycemia or hyperinsulinemia(11). Surprisingly when TEN is administered chronically in unstressed animals, which should activate the “portal signal”, NHGU is not any greater than that seen with TPN alone (1). This suggests that the “portal signal” is not effective chronically.

One explanation for the failure of TEN to further enhance NHGU is that the “portal signal” is time dependent and may only be effective when TEN is initiated. All of
the studies which have demonstrated an enhancement of NHGU by the enteral route have been done in less than 4 hours. Since the “portal signal” is very fast in enhancing NHGU(10), the beneficial effect of enteral glucose delivery may subside over time. A second possibility is that the unique metabolic fate of glucose (i.e. hepatic lactate release rather than hepatic glycogen deposition) during chronic nutritional support (TPN or TEN) diminishes the effectiveness of the “portal signal”. The primary fate enhanced by acute enteral glucose delivery is glycogen synthesis(20). In contrast the primary metabolic fate of NHGU in the adapted state is lactate release (1). It is possible that continued benefit of the portal signal requires further activation of glycolysis, which is not a target of the “portal signal”. A third possibility is that other nutrients in the nutritional support block the effect of the “portal signal”. Acute delivery of amino acids into the portal vein, but not into a peripheral vein, impairs the effect of the “portal signal”(15).

The dependency of the time course of hepatic adaptation on the route of nutrient delivery is unknown. Since enteral delivery of non-glucose nutrients can impair the portal signal, this may explain the failure of chronic TEN to further enhance NHGU. It is possible if chronic enteral glucose delivery (i.e. “portal signal” activation) is combined with parenteral delivery of the remaining nutrients it will be more effective than chronic parenteral delivery of all nutrients. Thus the aims of the study were 1) to examine the influence of the route of nutrient delivery on the time course of the hepatic adaptation during the first 8 hours of nutrient delivery, 2) to determine if chronic activation of the “portal signal” can enhance the adaptive response to TPN.
Methods

Animal preparation. Twenty seven female non-pregnant mongrel dogs (22±1 kg) were fed standard Kal-Kan meat (Vernon, CA) and Purina Lab Canine Diet #5006 (Purina Mills, St. Louis, MO) once daily and had free access to water. The composition of the diet based on dry weight was 52% carbohydrate, 31% protein, 11% fat, and 6% fiber. Dogs were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care International guidelines. The Vanderbilt University Medical Center Animal Care Committee approved the protocols. The health of the animals was determined before surgery and before administration of nutritional support as having: a good appetite (i.e., consumed at least ¾ of the daily ration), normal stools, hematocrit >35%, and leukocyte count < 18,000 mm³.

Experimental preparation. Fourteen to seventeen days before initiation of nutritional support blood sampling catheters were positioned in the left common iliac, portal and left common hepatic veins and the iliac artery(1). Two infusion catheters were placed in the inferior vena cava (IVC) for TPN, and an infusion catheter was inserted into the duodenum for TEN. Flow probes (Transonic Systems, Ithaca, NY) were positioned about the portal vein, hepatic artery, and right external iliac artery.

Experimental protocol. The first group of studies was performed on 42-hour fasted dogs in random order. The free ends of all catheters were exteriorized under local anesthesia (2% Lidocaine), and their contents were aspirated and flushed with saline. The free ends of the flow probes were also exteriorized and connected to a flow meter (Transonic Systems, Ithaca, NY). The dog was placed in a Pavlov harness for the duration of the study. The study consisted of a 90 min equilibration period, a 30 min
basal sampling (-30 to 0 min) period and an 8 hr nutrition infusion period. During the 30 minute basal sampling period blood samples were taken from the four sampling catheters (iliac artery, portal vein, hepatic vein and iliac vein) at –30, -15 and 0 min. TPN (n=6) or TEN (n=5), then was infused via IVC or duodenal catheter for 8-hours; the blood samples were taken hourly for the first 4-hours and every 30 minutes for the remaining 4 hrs.

Primed (44 and 27µCi) constant infusions (0.4 and 0.3 µCi/min) of [3-³H]- and [U-¹⁴C]glucose (New England Nuclear, Wilmington, DE), respectively, were infused into the IVC beginning 120 min after initiation of TPN or TEN. At the end of the study, the animals were killed with an overdose of pentobarbital sodium (Veterinary Lab, Lenexa, KS). Tissue samples from each of the seven liver lobes and a muscle (adductor magnus et brevis) were freeze-damped with Wallenburg clamps precooled in liquid nitrogen and were stored at -70º C until analysis. The entire liver was removed and weighed. TPN results have been reported(3).

In a second group of studies animals were placed on nutritional support for 5 days. Animals received either TPN (n=6), TEN (n=6) or a modified TPN (TPN+EG; n=4). In TPN+EG a portion (27.8 µmol/kg/min) of the glucose requirements given in TPN was given enterally and all non-glucose nutrients and the remaining portion of the glucose requirements (25 µmol/kg/min) were given parenterally. All three groups were performed in random order and the total amount of glucose infused were equal (52.8 µmol/kg/min). The results of TEN and TPN have been reported (1). On the morning of day 5 the catheters and flow probes were exteriorized as described above. After a 90 min equilibration period five samples were taken at 15 min intervals from each of the four sampling catheters. Biopsies were not taken because dogs were used for other studies.
Nutritional support. During the studies the sole caloric source was that infused either parenterally and/or enterally. The TPN or TEN was designed to be isocaloric, based on predicted resting energy expenditure (19). The components in the TPN and TEN were glucose, lipids, amino acids, saline (2.9 ml/kg/min), potassium phosphate (90 mg/kg/day), and a multivitamin supplement (MVI-12, Astra USA, Westborough, MA). Glucose (50% dextrose, Abbott) made up 75% of the nonprotein calories, and a fat emulsion (20% Intralipid, Baxter Healthcare, Deerfield, IL) constituted the remaining 25% of the energy requirements. Travasol (Baxter) was infused to supply basal nitrogen requirements (~12g protein/day), calculated with the formula $1.5 \times \text{body wt}^{0.67}$ (in kg). Nutrition solutions were prepared under sterile conditions. TPN or TEN was infused with calibrated infusion pumps (Harvard Apparatus, Holliston, MA; Dakmed Inc., Buffalo, NY).

Sample processing. Blood samples were placed in chilled tubes containing potassium EDTA (15mg). The collection and immediate processing of blood samples have been described previously (1, 4).

Analysis. Immunoreactive insulin and glucagon were assayed using modification of a radioimmunoassay kit from Linco Research [St. Louis, MO; intra-assay coefficient of variation (CV) 11% and 10%, respectively]. Cortisol was assayed with Diagnostic Products (Los Angeles, CA) RIA Kit (CV 12%).

Analysis of gluconeogenic metabolites (lactate, alanine, and glycerol) in blood was performed on an automated centrifugal analyzer (Monarch 2000; Instrumentation Laboratory, Lexington, MA) using a modification of the method of Lloyd et al(13). The
concentration of nonesterified fatty acids (NEFA) was determined spectrophotometrically (Wako Chemicals, Richmond, VA).

Calculations. The hepatic substrate load (Load In) was calculated as $A_s \times HABF + PV_s \times PVBF$, where $A_s$ and $PV_s$ represent the blood substrate concentrations in the iliac artery and portal vein, and $HABF$ and $PVBF$ represent blood flow in the hepatic artery and portal vein, respectively. Similarly, the substrate load leaving the liver (Load Out) was the product of $HV_s \times HBF$, in which $HV_s$ and $HBF$ represent the hepatic vein blood substrate concentration and total hepatic blood flow ($HABF + PVBF$) or plasma flow. Net hepatic substrate uptake was the difference between Load In and Load Out. Net hepatic substrate fractional extraction was calculated as the ratio of net hepatic substrate uptake and Load In. Net splanchnic substrate uptake was calculated as $(HABF + PVBF) * (A_s - H_s)$, where $A_s$ and $V_s$ represent the blood substrate concentrations in the iliac artery and hepatic vein. Plasma glucose was converted to blood glucose by a correction factor of 0.73(18). These equations were used to calculate net hepatic glucose, $^{14}$CO$_2$, lactate, alanine, glycerol, amino acid, and NEFA balances. To calculate organ NEFA balance plasma flow rather than blood flow was used; plasma flow was calculated by multiplying blood flow (hepatic artery, portal vein and total hepatic blood flow) by (1- hematocrit ratio).

Net hindlimb glucose uptake was calculated with the formula $(A_g - V_g) \times ABF$, where $A_g$ and $V_g$ represent blood glucose concentrations in the iliac artery and iliac vein and $ABF$ represents blood flow in the iliac artery. Net intestinal glucose uptake was calculated with the formula $(A_g - PV_g) \times PVBF$, where $A_g$ and $PV_g$ represent blood glucose concentrations in the iliac artery and portal vein and $PVBF$ represents blood flow.
in the portal vein. In TPN and TPN+EG net non-splanchnic glucose uptake was calculated as the difference between exogenous glucose infusion rate (GIR) given parenterally and net splanchnic glucose uptake. Since the splanchnic bed is the sole source of glucose for the peripheral tissues in TEN, net non-splanchnic glucose uptake was equal to net splanchnic glucose output.

Unidirectional intestinal glucose uptake was calculated as the ratio of intestinal $[^3\text{H}]$glucose uptake divided by the corresponding $[^3\text{H}]$ arterial glucose specific activity. Intestinal glucose production (i.e. absorption) was equal to the sum of net intestinal glucose output $[(PV_g-A_g)*PVBF]$ and unidirectional intestinal glucose uptake.

Hepatic conversion of glucose to CO$_2$ (hepatic glucose oxidation) was calculated as the net hepatic $^{14}\text{C}$CO$_2$ production rate divided by the hepatic $[^{14}\text{C}]$glucose precursor SA. The hepatic $[^{14}\text{C}]$glucose precursor SA was considered to be the $[^{14}\text{C}]$glucose SA in the blood flowing to the liver. Hepatic glucose storage was the sum of NHGU and total net gluconeogenic precursor uptake (lactate, amino acids and glycerol). Hepatic glycogen content in 42 h fasted dogs was assumed to equal 18 mg/g liver (8). Net deposition of glycogen from $^{14}\text{C}$ glucose in liver was calculated by dividing hepatic $[^{14}\text{C}]$ glycogen accumulation (dpm/g liver) by the average inflowing $[^{14}\text{C}]$glucose SA (dpm/mg).

Statistics. All values for the basal period are the average of data obtained at –30, –15 and 0 min. Unless otherwise stated, data for the experimental period are the mean of 7, 7.5 and 8 hrs. In the 5 day studies the mean data are the mean of the 5 samples taken on the day of the study. Statistical comparisons were made with two-way ANOVA followed by an $F$ test (SYSTA, Evanston, IL), and one-way ANOVA when comparing groups. $P < 0.05$ was regarded as significant.
Results

Response to an 8h hour nutrient infusion

Blood flow and Hormone concentrations. During the 8h nutrient infusion portal vein blood flow increased (P<0.05) in TEN, but not in TPN (Table 1). Hepatic arterial blood flow did not change in either group. Arterial plasma insulin concentrations increased to a greater extent (p<0.05) in TPN (44±8 to 234±35 pM) than in TEN (37±7 to 170±38 pM) 1 h after initiation of nutrient infusion. However they were similar by 8h (Fig. 1). The arterial plasma glucagon concentration decreased at the onset of nutritional support and remained suppressed for the duration of the study in both groups. (33±4 to 23±10 vs. 30±8 to 21±3 ng/L; basal to 8h; TPN vs.TEN).

Hepatic metabolism. The arterial blood glucose concentrations (Fig. 2) were similar during the basal period. During the first 8h of TPN or TEN they increased to a similar extent. The glucose infusion rate (GIR) was similar in both groups (52.4±0.3 vs. 54.3±0.2 μmol/kg/min; TPN vs. TEN).

Net hepatic glucose output (Fig. 3) was similar in the two groups during the basal period. During the nutrient infusion, the liver rapidly switched to a net glucose consumer. A stable rate of net hepatic glucose uptake (NHGU) was reached for 4 h after which NHGU gradually increased for the duration of the study. At 8h, NHGU increased by 50±16% and 46±35% in TPN and TEN groups as compared to NHGU seen at 4 h. Despite a lower hepatic glucose load because of the lower portal vein glucose concentration (Fig.2) in the TPN group, the NHGU was similar in the two groups. The net fractional hepatic glucose extraction in the two groups had a similar pattern as
NHGU. Hepatic glucose oxidation between 6 and 8 h was similar in the two groups (0.25±0.01 vs. 0.23±0.09 mg/kg/min, TPN vs. TEN).

The arterial blood lactate levels (Fig. 4) were similar during the basal period and increased after initiation of nutritional support. During the basal period the liver was a consumer of lactate in both groups. However with the onset of nutritional support a brisk but transient increase in net hepatic lactate release occurred. After 3 h net hepatic lactate release increased progressively for the duration of the study. At 8 h 33±9 % and 30±6 % of NHGU could be accounted for as lactate release (TPN and TEN).

Arterial blood alanine levels (Fig. 5) gradually increased to a similar extent in both groups during 8h of nutritional support. Net fractional hepatic alanine extraction remained unaltered for the first 4 hours of nutrient infusion, and then gradually decreased for the duration of the study.

Arterial blood glycerol levels (79±8 to 45±7 uM) and net hepatic glycerol uptake (1.8±0.2 to 1.2±0.2 µmol/kg/min) decreased in TEN, but were unaltered in TPN (85±9 to 90±29 µM and 1.8±0.2 to 1.6±0.4 µmol/kg/min). Net fractional hepatic glycerol extraction (0.63±0.02 to 0.64±0.02 vs. 0.61±0.01 to 0.58±0.02) did not change and was similar in both groups.

Arterial NEFA levels decreased markedly during TPN and TEN (962±65 to 256±48 vs. 799±100 to 200±31 µM). Since net fractional hepatic NEFA extraction did not change, net hepatic NEFA uptake decreased (3.5±0.9 to 0.5±0.1 vs. 3.4±1.6 to 0.7±0.2 µmol/kg/min).

The net retention of glucose carbon in the liver during 8h of nutritional support assuming a baseline of 18 mg/g liver increased to similar extent in both groups. (48±8
and 42±5 mg/g liver; TPN and TEN). Consistent with this both terminal liver glycogen content (54±5 vs. 58±1 mg/g liver, TPN vs. TEN) and 14C-determined glycogen synthesis (23±5 vs. 22±1 mg/g liver, TPN vs. TEN) were similar.

**Intestinal Metabolism:** The intestine was a net consumer of glucose prior to initiation of nutrient infusion and remained a consumer during the 8 h TPN infusion (Table 2). The intestine was a net producer of glucose (i.e. net glucose absorption) during TEN (Table 2). The net production of glucose by the intestine during the 8 h TEN could account for 67±5 % of the infused glucose during the first hour and 47±3% at 8 h.

Between 4 and 8 h unidirectional intestinal glucose uptake was higher (p<0.05) in the TPN group (4.3±0.7 vs. –0.1±0.0 µmol/kg/min; TPN vs. TEN). However, intestinal glucose production (i.e. absorption) was elevated (p<0.05) in TEN and was not different from zero in TPN (1±1 vs. 27±2 µmol/kg/min; TPN vs. TEN).

The release of lactate by the intestine during the basal period gradually decreased during the 8 h infusion. The intestine (Table 2) was a greater producer of alanine during the 8h TEN infusion; the increase could account for nearly all of the enterally delivered alanine (0.89 µmol/kg/min).

**Non-splanchnic and hindlimb metabolism.** Non-splanchnic glucose uptake (Non-SGU) increased during nutrient infusion in both groups. However, Non-SGU was significantly lower in TEN (Fig 6). Consistent with the rise in Non-SGU, Net hindlimb glucose uptake increased in TPN (8±4 to 58±10 µmol/min) and TEN (10±4 to 45±11 µmol/min). However we could not detect a blunted rise in hindlimb glucose uptake in TEN. Net fractional hindlimb glucose extraction increased similarly in the two groups (0.03±0.01 to 0.07±0.01 vs. 0.02±0.01 to 0.06±0.01; TPN vs. TEN). However, net
fractional hindlimb lactate extraction decreased (0.16±0.04 to 0.04±0.03 vs. 0.07±0.03 to 0.01±0.03; TPN vs. TEN) in the two groups.

Response to 5 days of nutrient infusion

Blood flow and Hormone concentrations. In animals receiving 5 days of nutritional support hepatic artery and portal vein blood flow were not significantly different among TPN, TEN and TPN+EG (Table 1). Arterial insulin concentrations were higher in TEN (p<0.05 vs. TPN) and lower (p<0.05 vs. TPN) in TPN+EG (96±6, 144±30 and 58±14 pM; TPN, TEN and TPN+EG). Arterial glucagon levels were higher (p<0.05) in TEN than TPN (28±5, 44±4 and 30±8 ng/L).

Hepatic metabolism. After 5 days of nutritional support NHGU was higher (~2-fold) in all groups than that seen after 8 h of nutritional support. Arterial glucose concentration and NHGU were not significantly influenced by the route of nutrient delivery. However net hepatic fractional glucose extraction was lower in TEN than TPN and this decrease was absent when only glucose was given by the enteral route (TPN+EG; Fig. 7).

After 5 days of nutritional support arterial blood lactate concentrations were 1.5±0.2, 1.3±0.2 and 1.3±0.2 mM in TPN, TEN and TPN+EG, respectively. Net hepatic lactate release was markedly increased (34±6, 31±4 and 44±1 µmol/kg/min; ~3-fold; p<0.05) as compared to that seen after 8 h. Net hepatic lactate release accounted for 75±12, 71±9 and 89±5% (TPN, TEN and TPN+EG) of NHGU. Arterial blood alanine concentrations (509±51, 532±55 and 616±46 µM) were higher (p<0.05) than that seen after 8 h of nutritional support. Net hepatic alanine uptake (1.4±0.3, 1.4±0.4 and 1.2±0.5 µmol/kg/min) and fractional extraction (0.08±0.02, 0.08±0.03 and 0.06±0.03) were not
affected by the route of nutrient delivery but were lower (p<0.05) that that seen after 8h of nutritional support.

After 5 days of nutritional support arterial glycerol concentrations (81±20, 46±3 and 49±3 µM) and net hepatic glycerol uptakes (1.4±0.4, 0.8±0.1 and 1.0±0.1 µmol/kg/min) were lower when nutrients were given enterally. Net hepatic fractional glycerol extraction (0.53±0.06, 0.54±0.02 and 0.64±0.03) was higher in TPN+EG. NEFA concentration (322±57, 215±26 and 244±36 µM) and net hepatic NEFA uptake (0.7±0.5, 0.2±0.1 and 0.4±0.4 µmol/kg/min) remained suppressed in all groups.

**Intestinal Metabolism:** The intestine was a net consumer of glucose after 5 days of TPN infusion (Table 2). The intestine was a net producer of glucose during TEN and TPN+EG infusion (Table 2). After 5 days of TEN and TPN+EG net intestinal glucose output could account for 72±9 and 65±8% (TEN and TPN+EG) of the glucose infused via the enteral route. The intestine (Table 2) was a greater producer of alanine after TEN than TPN; the increase could account for nearly all of the enterally delivered alanine (0.89 µmol/kg/min).

**Non-splanchnic metabolism.** Non-splanchnic glucose uptake (Non-SGU) increased during nutrient infusion in all groups. Non-SGU was significantly lower in TEN and TPN+EG (25.9±3.3, 16.1±3.9 and 19.1±0.4 µmol/kg/min; TPN, TEN and TPN+EG).
Discussion

The liver undergoes a profound adaptation to TPN to become a major site of glucose disposal during chronic (>5 days) nutritional support (1). Interestingly, the majority (75%) of the glucose carbon removed by the liver is released as lactate and the route of nutrient delivery does not influence the long term adaptive response (1). Our studies compared the early time course of this response to TPN and TEN. After 4hrs of TPN the liver switched to a more efficient consumer of glucose and by 8 hrs of TPN and TEN ~30% of the glucose taken up was released as lactate. While the efficiency of glucose disposal by the liver increased progressively, the rate of glycogen accretion remained constant over the 8-hour study. Surprisingly, TEN did not enhance the speed of this adaptation or alter the metabolic fate of glucose. Interestingly, delivery of a portion of the glucose requirements during chronic TPN via the enteral route did not alter NHGU, but decreased overall insulin requirements.

During the first 3 hours NHGU was not greater during TEN than during TPN. Previous work suggested that NHGU should be higher because portal glucose delivery activates the “portal signal” (20). In TPN the hepatic response was consistent with previous studies when only glucose was infused. Moore et al (16) demonstrated that, after 3 hours of a continuous infusion of glucose (55.6 µmol/kg/min), NHGU (6.1±0.2 µmol/kg/min) and net hepatic fractional glucose extraction (0.03±0.01) were similar to that seen in our study (5.8±2.2 µmol/kg/min and 0.03±0.01) at 3 h. While NHGU increased progressively during the 8 h infusion of TEN, NHGU (8.6±3.9 µmol/kg/min) and fractional extraction (0.03±0.01) were not greater than with TPN. Based upon previous work(16) and the higher hepatic glucose load, NHGU and fractional extraction
should have been much higher (~14µmol/kg/min and ~0.07) in TEN. One possible explanation for the failure of the “portal signal” to enhance NHGU is that amino acids in the TEN impaired the ability of the “portal signal” to augment NHGU. Interestingly, portal, but not peripheral, delivery of gluconeogenic amino acids impairs the “portal signal” (15). This would explain why amino acids in TPN did not impair NHGU. An additional explanation is that the capacity of the liver to take up and phosphorylate glucose has reached a maximum. This could explain why the portal signal may not be able to augment NHGU further. However both fructose induced-translocation of glucokinase and acute increases in glucose can markedly increased NHGU in the adapted setting(2, 5). Thus it is unlikely that glucose phosphorylation capacity is limiting.

The time course of hepatic adaptation to nutritional support was not influenced by the route of nutrient delivery. Within five hours of initiating nutritional support the efficiency of NHGU began to progressively increase for the remaining 3 hours irrespective of the route of nutrient delivery. Net hepatic fractional extraction of glucose increased gradually from 0.03 to 0.08 over the 3-hour period, despite gradual decreases in arterial glucose concentration and hepatic glucose load. A similar response was seen in TEN where fractional extraction rose from 0.04 to 0.07 over the same time period.

The hepatic adaptation to nutritional support is not complete after 8 hrs. After 8 h of nutritional support net hepatic fractional glucose extraction was 0.08 and 30% of the glucose taken up by the liver was released as lactate. Based upon our 5 day studies net hepatic fractional extraction of glucose can be greater than 0.15, a 2-fold increase over that seen at 8 h (1). Moreover greater than 75% of the glucose is converted to lactate after 5 days, a 3-fold increase over that seen at 8h. Our recent work suggests it takes less
than 24 hours to achieve the high efficiency of NHGU and fractional glucose extraction seen after 5 days of TPN(3).

The fraction of NHGU diverted to glycogen gradually decreased as the duration of TPN and TEN infusion increased. Despite glycogen synthesis being the primary fate of glucose disposal during the first 4 hrs of TPN and TEN, its rate of accretion did not increase as NHGU increased. In fact the slope of the relationship between accumulated net carbohydrate storage and time is linear for nearly 6 hrs. Thus any additional carbon taken up was diverted to alternative pathways such lactate release. Interestingly TEN did not augment the rate of accumulation of glycogen; total hepatic glycogen content was identical after TPN and TEN. The gradual shift of glucose carbon to other metabolic pathways occurred prior to reaching a stable glycogen level. This suggests that either the controller of glucose entry (presumably glucokinase) is gradually augmented to exceed glycogen synthetic capacity or pathways downstream of G-6P (e.g. phosphofructokinase) are gradually activated to facilitate G-6P metabolism, thus lowering the total resistance for glucose entry.

Chronic activation of the “portal signal” decreased insulin requirements during TPN. NHGU and net hepatic fractional glucose extraction were not higher in TPN+EG than TPN; however arterial insulin levels were 40% lower in TPN+EG. The arterial-portal glucose gradient in TPN+EG was large enough to maximally activate the portal signal (1.0±0.1 mM)(21). As long as insulin is present at concentrations at or above basal, the ability of the portal signal to augment NHGU acutely (†~2 mg/kg/min) is independent of the insulin levels(18). If the portal signal can persist chronically to sustain NHGU, the insulin concentration required to dispose of the entire exogenous
glucose load can be lowered. Generally with enteral glucose delivery hepatic glucose load is higher than when the same amount is glucose is delivered into a peripheral vein, thus favoring an increase in NHGU. Given the comparable net hepatic fractional extraction of glucose in TPN and TPN +EG, an increase in NHGU was expected in TPN+EG. However the slightly lower arterial glucose levels during TPN+EG offset the higher portal vein glucose levels, equating the hepatic glucose loads and therefore NHGU.

Acute activation of the portal signal is known to enhance insulin secretion(6), yet chronic activation of the portal vein lowered insulin levels. The lower arterial glucose levels likely contributed to the lower insulin levels. Consistent with previous studies only about 70% of the glucose given enterally is absorbed as glucose (8, 17); the remaining 30% is metabolized by the intestine. A failure to absorbed 100% of the enterally delivered glucose as glucose is likely not the sole explanation for the lower insulin concentration in TPN+EG. In TEN 70% of the glucose is absorbed and insulin levels are higher than TPN and TPN +EG. As already discussed the portal signal was ineffective in facilitating NHGU in TEN, it is likely that activation of the “portal signal” diminished the overall insulin requirements in TPN+EG. Interestingly in head trauma patients(23) insulin requirements are lower when patients are given TEN as compared to TPN, however this decrease may be due to enhanced endogenous insulin secretion.

The ability of the “portal signal” to limit non-splanchnic glucose uptake persists in TEN. During TPN non-splanchnic glucose uptake remained elevated for the duration of the study. In contrast, in TEN non-splanchnic glucose uptake gradually decreased during the 8 h infusion, despite similar glucose and insulin concentrations as in TPN.
Even after 5 days of TEN and TPN+EG non-splanchnic glucose uptake was decreased. This is consistent with the known inhibitory effect of the “portal signal” on glucose uptake by peripheral tissues (9, 20). Muscle is one of the targets (9). However we were unable to detect a corresponding decrease in hind limb glucose uptake. The lower glucose disposal by peripheral tissues during TEN or TPN+EG is not due to differences in fatty acid availability. NEFA were markedly suppressed in all groups. It is interesting that delivery of amino acids into the portal vein impaired the ability of the portal signal to facilitate NHGU, but is unable to impair its ability to restrain peripheral glucose uptake(15). This amino acid effect on the “portal signal” is consistent with the persistent attenuation of non-splanchnic glucose uptake in TEN despite an apparent defect in the portal signal’s ability to facilitate NHGU.

In summary irrespective of the route of nutrient delivery the adaptation to nutritional support by the liver begins within 8 hours. However it does not attain the highly efficient glucose-consuming organ seen after 5 days of nutritional support. This gradual shift to a more efficient glucose-consuming state begins approximately 5 hrs after initiation of nutritional support. Surprisingly, while TEN does not enhance the adaptive response to TPN, chronic activation of the “portal signal” by delivering a portion of the glucose requirements enterally markedly decreased the insulin requirements during TPN. It is thus possible that in patients receiving TPN, if a small portion of the glucose were given enterally this would limit the insulin requirement and the risk of hyperglycemia that is commonly seen with stressed patients.
Acknowledgements:

This work was supported by NIH grant DK-43748 (PI: Owen McGuinness), Clinical Nutrition Research Unit Grant P30-DK-26657 and the Vanderbilt Diabetes Training and Research Center Grant (DK-20593). We are grateful for expert technical assistance of Wanda Snead and Angie Penaloza in the Vanderbilt DRTC hormone core laboratory.
Table 1. Hepatic artery, portal vein, and total hepatic blood flow in dogs receiving nutritional support either for 8 hours or 5 days

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>TEN or TPN infusion (min)</th>
<th>5 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>120</td>
</tr>
<tr>
<td>Hepatic artery flow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEN</td>
<td>6.0±1.1</td>
<td>5.5±1.4</td>
<td>6.1±1.1</td>
</tr>
<tr>
<td>TPN</td>
<td>6.5±0.9</td>
<td>8.0±1.7</td>
<td>6.6±2.0</td>
</tr>
<tr>
<td>TPN+EG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portal vein flow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEN</td>
<td>25.1±1.8</td>
<td>31.6±0.7*</td>
<td>28.7±1.5*</td>
</tr>
<tr>
<td>TPN</td>
<td>23.9±3.2</td>
<td>22.2±1.7</td>
<td>20.7±2.8</td>
</tr>
<tr>
<td>TPN+EG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total hepatic flow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEN</td>
<td>31.1±2.8</td>
<td>37.1±2.0</td>
<td>34.8±2.5</td>
</tr>
<tr>
<td>TPN</td>
<td>30.4±3.7</td>
<td>30.2±3.0</td>
<td>27.3±4.6</td>
</tr>
<tr>
<td>TPN+EG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are from 42-h-fasted dogs during a basal period and during a 480 min of isocaloric nutrition infusion given enterally (TEN; n=5) or parenterally (TPN; n=6). A separate group of animals received TPN, TEN, or TPN with a portion of the glucose given via the enteral route (TPN+EG) for five days. Data are the mean of all the sampling times on the fifth day of infusion. Blood flow are in ml/kg/min. Data are expressed as means±SEM. * significantly different from basal period of TEN group.
Table 2. Net intestinal glucose, lactate, and alanine output in dogs receiving nutritional support either for 480 min or 5 days

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>60</th>
<th>120</th>
<th>240</th>
<th>300</th>
<th>420</th>
<th>480</th>
<th>5 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TEN</strong></td>
<td>-1.0±0.7</td>
<td>35.0±2.6*</td>
<td>31.6±4.8*</td>
<td>29.2±3.3*</td>
<td>25.6±1.8*</td>
<td>26.3±1.9*</td>
<td>24.8±1.5*</td>
<td>36.8±4.1*</td>
</tr>
<tr>
<td><strong>TPN</strong></td>
<td>-2.1±0.8</td>
<td>-0.2±1.3</td>
<td>0.2±2.9</td>
<td>-1.4±1.8</td>
<td>-4.4±0.3</td>
<td>-1.7±0.6</td>
<td>-3.4±1.1</td>
<td>-3.2±0.4</td>
</tr>
<tr>
<td><strong>TPN+EG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>18.1±2.1*</td>
</tr>
<tr>
<td><strong>Glucose</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lactate</strong></td>
<td>1.3±0.5</td>
<td>1.8±0.9</td>
<td>0.7±0.4</td>
<td>1.4±0.3</td>
<td>0.7±0.5</td>
<td>-0.3±0.4</td>
<td>0.1±0.9</td>
<td>0.3±0.5</td>
</tr>
<tr>
<td><strong>TPN</strong></td>
<td>2.4±0.3</td>
<td>0.2±0.7</td>
<td>0.9±0.6</td>
<td>0.1±0.8</td>
<td>-0.2±0.2</td>
<td>-0.1±0.5</td>
<td>-0.5±0.6</td>
<td>-2.5±0.9</td>
</tr>
<tr>
<td><strong>TPN+EG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-2.9±1.7</td>
</tr>
<tr>
<td><strong>Alanine</strong></td>
<td>0.8±0.2</td>
<td>2.1±0.2*</td>
<td>1.7±0.3*</td>
<td>1.8±0.3*</td>
<td>1.5±0.2*</td>
<td>1.4±0.3*</td>
<td>1.4±0.2*</td>
<td>1.6±0.2*</td>
</tr>
<tr>
<td><strong>TPN</strong></td>
<td>0.9±0.2</td>
<td>1.0±0.2</td>
<td>0.8±0.4</td>
<td>0.8±0.2</td>
<td>0.7±0.1</td>
<td>0.8±0.1</td>
<td>0.7±0.2</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td><strong>TPN+EG</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.8±0.1</td>
</tr>
</tbody>
</table>

Values are from 42-h-fasted dogs during a basal period and during a 480 min of isocaloric nutrition infusion given enterally (TEN; n=5) or parenterally (TPN; n=6). A separate group of animals received TPN, TEN, or TPN with a portion of the glucose given via the enteral route (TPN+EG) for five days. Data are the mean of all the sampling times on the fifth day of infusion. All output rates are in µmol/kg/min. Data are expressed as means±SEM. * significantly different from TPN group.
Figure 1. Arterial plasma insulin concentrations in 42-h-fasted dogs during the basal period and an infusion period of total enteral (TEN) and parenteral nutrition (TPN) for 8 hours. Data are expressed as means±SEM.
Figure 2. Arterial blood glucose concentrations and hepatic glucose load in 42-h-fasted dogs during the basal period and an infusion period of total enteral (TEN) and parenteral nutrition (TPN) for 8 hours. Data are expressed as means±SEM.
Figure 3. Net hepatic glucose uptake and net fractional hepatic glucose extraction in 42-h-fasted dogs during the basal period and an infusion period of total enteral (TEN) and parenteral nutrition (TPN) for 8 hours. Data are expressed as means±SEM.
Figure 4. Arterial blood lactate concentrations and net hepatic lactate release in 42-h-fasted dogs during the basal period and an infusion period of total enteral (TEN) and parenteral nutrition (TPN) for 8 hours. Data are expressed as means±SEM.
Figure 5. Arterial blood alanine concentrations, net hepatic alanine uptake, and net fractional hepatic alanine extraction in 42-h-fasted dogs during the basal period and an infusion period of total enteral (TEN) and parenteral nutrition (TPN) for 8 hours. Data are expressed as means±SEM.
Figure 6. Non-splanchnic glucose uptake (Non-SGU) in 42-h-fasted dogs during the basal period and an infusion period of total enteral (TEN) and parenteral nutrition (TPN) for 8 hours. Data are expressed as means±SEM.
Figure 7. Arterial plasma glucose concentration, net hepatic glucose uptake and net fractional hepatic glucose extraction in dogs receiving total enteral (TEN) or parenteral (TPN) nutrition or TPN with a duodenal glucose infusion (TPN+EG) for 5 days. Data are expressed as means±SEM.
References


