Effects of enteral carbohydrates on de novo lipogenesis in critically ill patients[1–3]

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ABSTRACT

Background: Conversion of glucose into lipid (de novo lipogenesis; DNL) is a possible fate of carbohydrate administered during nutritional support. It cannot be detected by conventional methods such as indirect calorimetry if it does not exceed lipid oxidation.

Objective: The objective was to evaluate the effects of carbohydrate administered as part of continuous enteral nutrition in critically ill patients.

Design: This was a prospective, open study including 25 patients nonconsecutively admitted to a medicosurgical intensive care unit. Glucose metabolism and hepatic DNL were measured in the fasting state or after 3 d of continuous isoenergetic enteral feeding providing 28%, 53%, or 75% carbohydrate.

Results: DNL increased with increasing carbohydrate intake ($\bar{x}$ ± SEM: 7.5 ± 1.2% with 28% carbohydrate, 9.2 ± 1.5% with 53% carbohydrate, and 19.4 ± 3.8% with 75% carbohydrate) and was nearly zero in a group of patients who had fasted for an average of 28 h (1.0 ± 0.2%). In multiple regression analysis, DNL was correlated with carbohydrate intake, but not with body weight or plasma insulin concentrations. Endogenous glucose production, assessed with a dual-isotope technique, was not significantly different between the 3 groups of patients (13.7–15.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$), indicating impaired suppression by carbohydrate feeding. Gluconeogenesis was measured with [^{13}C]bicarbonate, and increased as the carbohydrate intake increased (from 2.1 ± 0.5 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ with 28% carbohydrate intake to 3.7 ± 0.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ with 75% carbohydrate intake, $P < 0.05$).

Conclusion: Carbohydrate feeding fails to suppress endogenous glucose production and gluconeogenesis, but stimulates DNL in critically ill patients. *Am J Clin Nutr* 2000;72:940–5.

KEY WORDS Glucose production, gluconeogenesis, lipogenesis, insulin resistance, enteral nutrition, critically ill patients, de novo lipogenesis

INTRODUCTION

Endogenous glucose production and plasma glucose concentrations increase during stress induced by trauma, surgery, or infection or sepsis (1). Furthermore, administration of parenteral or enteral carbohydrate fails to suppress glucose production in critically ill patients, indicating hepatic resistance to glucose, insulin, or both (2–6). Simultaneously, endogenous protein breakdown is increased and is poorly suppressed by enteral or parenteral nutrients in critically ill patients unless large amounts of exogenous glucose or glucose-insulin mixtures are used (7, 8).

Administration of glucose in excess of energy requirements is well known to promote net de novo lipogenesis (DNL) in both critically ill patients (9, 10) and healthy subjects (11). This leads to increased carbon dioxide production and thermogenesis and hence may have deleterious effects in patients with compromised pulmonary function (10). In these studies, indirect calorimetry was used to assess the rate of whole-body net DNL (ie, fat synthesis in excess of concomitant fat oxidation at the whole-body level) (12). More recently, a novel isotopic approach was developed to assess hepatic DNL in vivo even if it did not exceed whole-body fat oxidation (13, 14). With the use of this approach, we found recently that DNL was stimulated in critically ill patients receiving isoenergetic total parenteral nutrition when carbohydrates made up 75% of the energy supply. In contrast, DNL remained low in critically ill patients receiving an isoenergetic, low-carbohydrate diet (15% of total energy as total parenteral nutrition). These results suggest that DNL was stimulated by exogenous carbohydrate in critically ill patients, as was observed in healthy subjects (15). In addition, the route of carbohydrate administration (ie, parenteral or enteral nutrition) may affect DNL differently.

Our previous observation of carbohydrate-induced stimulation of DNL in critically ill patients (5) appears to be at odds with the well-known hepatic insulin resistance of this group (16). This suggests that insulin resistance affects hepatic glucose production and DNL differently. We therefore measured DNL, endogenous glucose production, and gluconeogenesis simultaneously in critically ill patients requiring continuous enteral nutrition. Patients were randomly assigned to receive isonitrogenous,

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TABLE 1
Patient characteristics[1]

Patient group and sex	Age	Weight	Height	Diagnosis
	y	*kg*	*cm*	
EN-75%				
M	23	76	175	Trauma
F	45	60	163	Urinary sepsis
M	61	77	173	Trauma
M	50	90	177	Trauma
M	67	65	173	Abdominal sepsis
M	27	75	175	Trauma
F	70	50	149	Cardiac failure, postcardiac surgery
EN-53%				
M	46	58	175	Brain injury
F	68	54	154	Esophagectomy for carcinoma
M	45	72	164	Subarachnoid hemorrhage
M	33	84	182	Thoracic trauma
M	65	63	178	Esophagectomy for carcinoma
M	56	80	175	Ileus
M	25	70	178	Subdural hematoma
EN-28%				
M	74	101	169	Pulmonary sepsis
F	67	110	155	Stroke
F	68	60	160	Cardiac failure, postmyocardial infarction
F	68	70	156	Cardiac failure, postmyocardial infarction
M	61	56	174	Cardiac failure, postmyocardial infarction
F	59	105	172	Respiratory failure, chronic obstructive pulmonary disease
F	48	50	160	Respiratory failure, chronic obstructive pulmonary disease
Fasted				
M	68	70	172	Oropharyngeal abcess
M	33	75	170	Coronary bypass
M	67	70	167	Trauma
M	62	64	161	Resection of thoracic aneurysm
M	52	72	167	Trauma

[1] The enteral nutrition solutions provided 75%, 53%, or 28% of energy as carbohydrate; the fasted group received an infusion of [1-^{13}C]acetate after they had fasted for an average of 28 h.

isoenergetic, continuous enteral nutrition providing 28%, 53%, or 75% carbohydrate. In an additional group of patients, DNL was measured after a 24–36-h fast.

SUBJECTS AND METHODS

Subjects

Twenty-five patients (18 men, 7 women) hospitalized in a surgical or medical intensive care unit were selected to take part in this study. Their main physical characteristics and clinical conditions are shown in **Table 1**. None had known preexisting diabetes mellitus or digestive disorders. The experimental protocol was approved by the Ethical Committee of Lausanne University School of Medicine and informed, written consent was obtained from the patients or their close relatives.

Experimental protocol

Each patient was assigned to 1 of 4 groups. In the first 3 groups, indirect calorimetry was done at inclusion after the patients had fasted for ≥12 h. This assessment was performed over 30–60 min under resting conditions, with a Deltatrac II monitor (Datex Instruments, Helsinki). Thereafter, continuous enteral nutrition was started through a nasogastric or jejunal feeding tube at a rate providing 50% of the measured energy expenditure over the first 24 h. On the morning of day 2, the infusion rate was increased to cover 100% of energy expenditure. Indirect calorimetry was repeated in the morning of day 3 and the nutrient infusion rate was readjusted to cover 100% of energy requirements during the next 24 h. On the morning of day 4, a primed (2 $\mu g \cdot kg^{-1} \cdot$ mmol fasting glucose^{-1}), continuous (2 $\mu g \cdot kg^{-1} \cdot min^{-1}$) intravenous infusion of [$^2H_7$]glucose (Masstrace, Woburn, MA) was started at 0800 and continued until 1200. Concomitantly, a primed (1 mmol), continuous (0.1 mmol/min) intravenous infusion of $NaH^{13}CO_3$ (Masstrace) was administered. An infusion of [6,6-^{2}H]glucose was administered through the nasogastric tube together with nutrients to achieve an isotopic enrichment of exogenous carbohydrate of 2.0 mole percent excess. At 1200, these intravenous tracer infusions were replaced by an infusion of [1-^{13}C]acetate delivered at a rate of 0.5 g/h to measure fractional DNL. This intravenous infusion was continued until 2200. Blood samples were obtained at 0800 (basal) and at 1100 and 1200 to monitor plasma [$^2H_7$]- and [^{13}C]glucose, and at 2000, 2100, and 2200 to monitor the isotopic enrichment of VLDL palmitate.

One group of patients received enteral nutrition providing 28% of the energy as carbohydrates (EN-28%, Pulmocare; Abbott Ross Co, Columbus, OH), another group received enteral nutrition pro-

TABLE 2
Composition of the 3 enteral nutrition solutions[1]

	EN-75%	EN-53%	EN-28%
		g/L	
Carbohydrate	307.6	200.0	105.6
Lipids	17.1	50.0	46.0
Protein	64.6	62.5	62.5

[1] The enteral nutrition solutions provided 75%, 53%, or 28% of energy as carbohydrate.

viding 53% of the energy as carbohydrates (EN-53%, Ensure; Abbott Ross), and a third group received enteral nutrition providing 75% of the energy as carbohydrate (EN-75%; prepared by the Abbott Ross Co using the same ingredients as in Pulmocare). The composition of the 3 nutrient mixtures is shown in **Table 2**.

[1-^{13}C]Acetate alone was infused to monitor DNL between 0800 and 1800 in a fourth group of patients (fasted group) who had fasted for an average of 28 h.

Analytic procedures

For glucose isotope determinations, plasma was deproteinized with 3% perchloric acid and partially purified over sequential cation anion exchange resins (AG 50W-X8 and AG 1-X8; Bio-Rad, Richmond, CA). For determination of [$^2H_7$]- and [6,6-^{2}H]glucose concentrations, pentacetyl glucose derivates were analyzed by gas chromatography–mass spectrometry (GC-MS) (GC 5890, MS 5971; Hewlett-Packard Co, Palo Alto, CA) in chemical ionization mode with selective monitoring at mass-to-charge ratios of 331, 333, and 338. To determine plasma [13C]glucose abundance, partially purified plasma samples were further purified with HPLC and analyzed by combustion with isotope ratio mass spectrometry (Roboprep CN-Tracermass; Europa Scientific, Crewe, United Kingdom) (17).

The palmitic acid methyl esters prepared from the VLDL-triacylglycerol were shipped to the University of California at Berkeley. GC-MS (MS 5973, GC 6890; Hewlett-Packard Co) with a 20-m DB1 column was used to quantify the molecular ions of methyl palmitate (mass-to-charge ratio: 270–272) by using electron-impact ionization in the selected ion-monitoring mode (13).

Plasma glucose was measured with a glucose analyzer (model II; Beckman Instruments, Fullerton, CA). Plasma fatty acid concentrations were measured with a calorimetric method by using a kit from Wako (Freiburg, Germany). Plasma insulin (kit from Biochem Immunosystem GmbH, Freiburg, Germany), glucagon (kit from Linco Research, St Charles, MO), and cortisol (kit from DPC, Los Angeles) were measured with radioimmunoassays.

Glucose kinetics

Rates of glucose appearance and disappearance were calculated from plasma [$^2H_7$]glucose enrichments. Because the isotopic enrichment of plasma glucose increased slightly between 180 and 240 min, non–steady state equations were used (18). A pool fraction of 0.75 and a distribution volume of 0.2 L glucose/kg were used for these calculations.

$$\text{Rate of exogenous glucose appearance} = (\text{rate of glucose appearance}) \cdot (\text{plasma [6,6-}^2\text{H]glucose enrichment at time 240 min} / [6,6\text{-}^2\text{H]glucose enrichment of enterally infused carbohydrate}) \quad (1)$$

Gluconeogenesis was estimated as (plasma [^{13}C]glucose)/(breath $^{13}CO_2$)·(rate of glucose appearance) (19). No correction factor was used for isotopic exchange in the Krebs cycle.

Fractional DNL was calculated from [^{13}C]VLDL-palmitate enrichment by the mass isotopomer distribution analysis technique, as described previously (13). The ratio of excess doubly labeled species to excess singly labeled species (EM2:EM1) in VLDL palmitate indicates the isotopic enrichment of the intrahepatic acetyl-CoA pool by application of probability logic based on the binomial expansion. The fractional contribution from DNL to the VLDL fatty acids was then calculated by their precursor-product relation. For this calculation, the isotopic enrichment of the intrahepatic acetyl-CoA pool was obtained from the binomial distribution of VLDL-palmitate mass isotopomers (13).

Statistics

Comparisons between groups were made by analysis of variance and unpaired *t* tests with Bonferroni correction. The relation between body weight, carbohydrate intake (g/h), and plasma insulin concentrations on one hand, and fractional DNL on the other hand, were assessed by multiple regression analysis. All results are expressed as means ± SEMs. All statistical analyses were performed by using STATVIEW 4.0 (Abacus Concepts, Berkeley, CA).

RESULTS

Plasma concentrations of glucose, fatty acids, and major glucoregulatory hormones in the 4 groups of patients studied are shown in **Table 3**. Plasma glucose and insulin concentrations were significantly higher in the 3 groups of patients who received enteral nutrition than in the acetate-infused, fasted group. There were no significant differences between the EN-28%,

TABLE 3
Hormones and substrate concentrations after 3 d of enteral nutrition[1]

Patient group	Glucose	Insulin	Glucagon	Fatty acids	Triacylglycerol	Cortisol
	mmol/L	*pmol/L*	*ng/L*	*mmol/L*	*mmol/L*	*nmol/L*
EN-75% (n = 7)	8.8 ± 0.9[2]	299 ± 61[2]	153 ± 23	0.247 ± 0.065[2]	1.59 ± 0.30	499 ± 117
EN-53% (n = 7)	7.9 ± 0.8[2]	344 ± 94[2]	113 ± 15	0.262 ± 0.067[2]	1.89 ± 0.30	429 ± 53
EN-28% (n = 7)	7.9 ± 0.7[2]	209 ± 54[2]	228 ± 39	0.730 ± 0.117[3]	2.04 ± 0.20	460 ± 72
Fasted (n = 5)	6.2 ± 0.8[3]	80 ± 21[3]	187 ± 33	0.609 ± 0.087[3]	2.42 ± 0.57	616 ± 89

[1] $\bar{x}$ ± SEM.The enteral nutrition solutions provided 75%, 53%, or 28% of energy as carbohydrate; the fasted group received an infusion of [1-^{13}C]acetate after they had fasted for an average of 28 h.

[2] Significantly different from the fasted group, $P \leq 0.05$.

[3] Significantly different from EN-75%, $P < 0.02$.

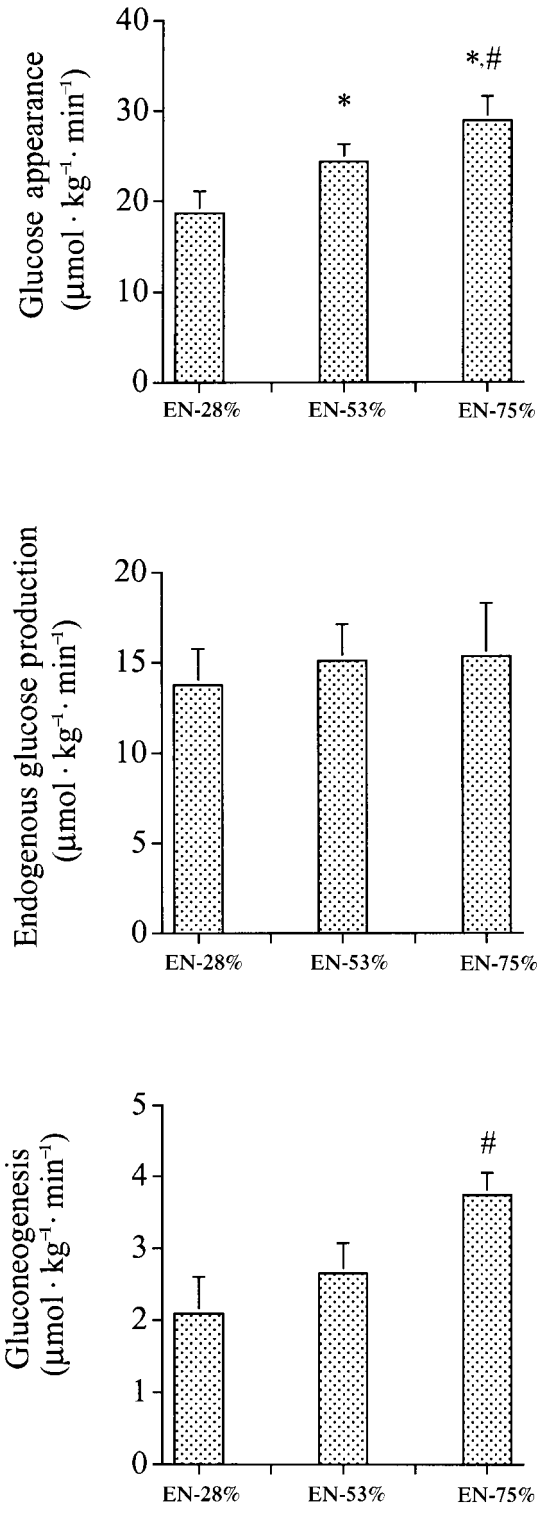

FIGURE 1. Mean (±SEM) glucose appearance, endogenous glucose production, and gluconeogenesis in patients who received isoenergetic, isonitrogenous continuous enteral nutrition providing 28% (EN-28%; $n = 7$), 53% (EN-53%; $n = 7$), or 75% (EN-75%; $n = 7$) of energy as carbohydrate. *Significantly different from EN-28%, $P < 0.05$. #Significantly different from EN-53%, $P < 0.05$.

EN-53%, and EN-75% groups. Plasma fatty acid concentrations were significantly higher in the fasted and EN-28% groups than in the EN-53% and EN-75% groups.

Rates of glucose appearance were higher in the EN-53% and EN-75% groups than in the EN-28% group (**Figure 1**). Endogenous glucose production, however, was not significantly different between the 3 groups. Gluconeogenesis was 79% higher in the EN-75% group than in the EN-28% group.

DNL was not significantly different from zero in the fasted group. In the 3 groups fed enteral nutrition, DNL was higher in proportion to the amount of carbohydrate administered (**Figure 2**). On the basis of multiple regression analysis, carbohydrate intake was positively correlated with DNL ($t = 2.14$, $P < 0.05$). No correlations between body weight or plasma insulin concentrations and DNL were observed.

DISCUSSION

The major observation of our study was that fractional hepatic DNL in critically ill patients was highly dependent on the amount of carbohydrate administered enterally: DNL was virtually absent in patients who had fasted for an average of 28 h (fasted group), was modest in the EN-28% and EN-53% groups, and increased to 15–20% in the EN-75% group.

Whole-body, absolute rates of lipogenesis could not be determined for 2 reasons. First, a quantitative rate of hepatic DNL would have required the simultaneous measurement of the VLDL production rate, which was not performed in this study. Second, only hepatic DNL is assessed by monitoring the rate of isotope incorporation in VLDL-palmitate. Previous studies performed on isolated adipocytes indicated that adipose tissue lipogenesis may represent up to 40% of whole-body lipogenesis in nutritionally depleted patients during high-carbohydrate refeeding (20). More recently, it was observed with the combined use of indirect calorimetry and tracer methods that adipose tissue lipogenesis may account for a major portion of total fat synthesis in healthy subjects submitted to 4 d of massive overfeeding (21). Despite a >15-fold stimulation of hepatic fractional DNL by high-carbohydrate feeding in the present study, indirect calorimetry measurements (performed in 18 of the 21 patients receiving enteral nutrition) showed that nonprotein respiratory quotients did not exceed 1.0 (data not shown). This indicates that the amount of lipid newly synthesized from glucose was completely offset by concomitant oxidation of fat (14). It can therefore be concluded that DNL occurs during high enteral carbohydrate intake in critically ill patients when no excess energy is administered but is likely to remain quantitatively modest.

In a previous study we found that DNL was stimulated in critically ill patients receiving high-glucose total parenteral nutrition but not in patients receiving isoenergetic amounts of low-glucose, high-fat total parenteral nutrition (5). The estimates of DNL were comparable with those observed in the present study, indicating no major role of the route of substrate administration. Tumor necrosis factor α (TNF-α), interleukin 1, and interferon α were shown to stimulate lipogenic enzymes in liver cells in vitro (22) and in experimental animals in vivo (23) and may have played a role in stimulating hepatic DNL in our patients.

Our present data, however, suggest that TNF-α and other stress mediators that are known to be higher in critically ill patients than in healthy subjects are unable to stimulate DNL in the absence of nutritional factors, as was reported in experimen-

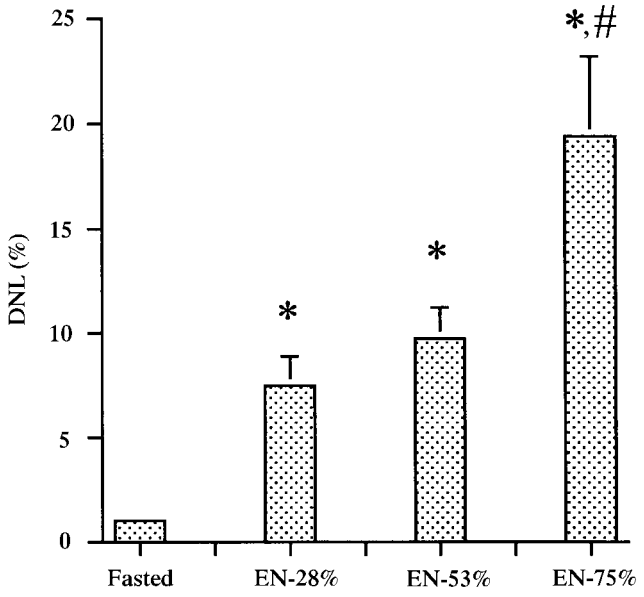

FIGURE 2. Mean (±SEM) fractional hepatic de novo lipogenesis (DNL) in critically ill patients who had fasted for an average of 28 h (n = 5) or who received isoenergetic isonitrogenous continuous enteral nutrition containing 28% (EN-28%; n = 7), 53% (EN-53%; n = 7), or 75% (EN-75%; n = 7) of energy as carbohydrate. *Significantly different from the fasted group, $P < 0.05$. #Significantly different from EN-28% and EN-45%, $P < 0.05$.

tal animals (23). DNL was virtually absent in fasted, critically ill patients. Furthermore, in the patients fed enteral nutrition solutions, DNL appeared to be essentially regulated by nutritional factors in a manner similar to that in healthy subjects. A stimulatory effect of a high-carbohydrate, isoenergetic diet on DNL was documented in healthy subjects (15).

Carbohydrate-induced stimulation of DNL in critically ill patients appears surprising given their well-known impaired suppression of glucose production in response to carbohydrate administration. There is ample evidence that glucose production is not adequately suppressed by parenteral glucose and insulin in critically ill patients (2–5). A more detailed description of glucose kinetics in the EN-28% and EN-75% groups was reported elsewhere, indicating that glucose production is also suppressed when carbohydrates are administered enterally in critically ill patients (6). Our present assessments of glucose kinetics corroborate and extend these earlier observations. Endogenous glucose production was not significantly different between the 3 enteral nutrition–fed groups tested, and was even superior to values observed in the fasted, healthy volunteers (24), indicating continuing endogenous glucose production despite large exogenous glucose loads in the EN-53% and EN-75% groups. In addition, gluconeogenesis was assessed semiquantitatively by the rate of [^{13}C]glucose synthesis during continuous infusion of [^{13}C]bicarbonate. This method is known to underestimate the actual rate of gluconeogenesis (19, 25). Our present observations, nonetheless, indicate that gluconeogenesis was still occurring after 3 d of continuous enteral nutrition. This finding is consistent with the results of a study by Newgard et al (26) showing that carbohydrate feeding inhibited net glucose production by inhibiting glucose-6-phosphatase, whereas glucose formed from gluconeogenesis was directed toward glycogen synthesis. Gluconeogenesis was modestly, although significantly, higher in the group receiving the highest dose of glucose. The reason for this stimulation of gluconeogenesis during high-carbohydrate feeding is unclear. Such stimulation may reflect a higher rate of cycling between glucose and lactate (Cori cycle) during high-carbohydrate loading. In support of this explanation is the observation that overfeeding stimulated Cori cycle activity and net glucose output in healthy volunteers (27).

Our observation that DNL was stimulated by dietary carbohydrate consumption while suppression of glucose production was impaired suggests that critical illness differentially affects the metabolic pathways involved in glucose production and lipogenesis. This implies a defective inhibition by insulin and glucose of phosphorylase, gluconeogenic enzymes, and glucose-6-phosphatase, but a normal insulin-induced activation of lipogenic enzymes. One possible explanation is that insulin resistance induced by stress mediators (eg, TNF-α, interleukins, and prostaglandins or leukotrienes) involves impaired suppression of glucose production but does not affect stimulation of lipogenesis. An alternative explanation is that some of the hepatic metabolic alterations stem from extrahepatic release of gluconeogenic substrates, such as amino acids and lactate from skeletal muscle or glycerol from higher lipolysis. According to this scheme, extrahepatic insulin resistance would be responsible for secondary liver insulin resistance due to increased gluconeogenesis. If that was true, our observation of normal DNL during [^{13}C]acetate infusion may actually represent fat synthesis from gluconeogenic precursors rather than from carbohydrate.

In conclusion, our present observations indicate that DNL, as assessed from [^{13}C]VLDL-palmitate synthesis during [^{13}C]acetate infusion, is stimulated by isoenergetic continuous enteral infusion of high-carbohydrate formulas in critically ill patients. This effect may be undesirable in patients with respiratory distress. The observation of DNL stimulation by carbohydrates in a group of patients who were simultaneously characterized by an impaired suppression of gluconeogenesis and glucose production in response to carbohydrate administration indicates that the glucose production and lipogenic pathways are differentially affected by critical illness. Increased release of extrahepatic gluconeogenic precursors may be primarily involved in both the maintenance of a high rate of endogenous glucose production and stimulation of lipogenesis by carbohydrate in critically ill patients.

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