〔Chiba Med J 74 : 393～399, 1998〕

〔Original Paper〕

Glucose Oxidation in Critically Ill Patients Measured with Stable Isotope and Indirect Calorimetry

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(Received June 22, 1998, Accepted July 1, 1998)

SUMMARY

Measurements of glucose oxidation, using a primed constant infusion of D-[U-^{13}C] glucose, and indirect calorimetry were made in parallel in critically ill patients. Validity of the measurement of glucose oxidation rate by the use of stable isotope in addition to indirect calorimetry was demonstrated.

Isotopic measurement and indirect calorimetry were performed preoperatively and on day 3 postoperatively in four patients who underwent surgery for esophageal carcinoma.

Priming doses of $NaH^{13}CO_3$ and D-[U-^{13}C]glucose were 0.32mg／kg and 0.32mg／kg, respectively. D-[U-^{13}C] glucose was then infused at an infusion rate of 0.004mg／kg／min. Concentration of glucose, free fatty acid (FFA) and ketones in plasma were also measured.

Plasma levels of FFA and ketones increased on day 3 following surgery when compared to preoperative values in all of the patients, indicating an increased mobilization and oxidation of fat in the critically ill state. Indirect calorimetry, however, demonstrated nonprotein respiratory quotient (RQ) above 1.0 on the 3rd postoperative day, suggesting that fat was being synthesized rather than being oxidized. On the other hand, isotopic measurement indicated decreased oxidation of glucose together with increased lipid oxidation in all four patients. These results from isotopic measurement were compatible with the serum levels of FFA and ketones. Increased recycling of energy substrates was suggested from the present study using indirect calorimetry.

Thus, isotopic measurement, in combination with indirect calorimetry, provides a useful information which can not be obtained from indirect calorimetry alone, and is essential for investigating the energy metabolism in critically ill patients.

Key words : Primed constant infusion, glucose oxidation, indirect calorimetry, stable isotope, critical illness

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千葉大学医学部外科学第一講座
1998年6月22日受付，1998年7月1日受理

I. Introduction

Efficacy of utilization of energy substrates has been reported to alter in pathophysiological situations, such as diabetes meritus[1, 2], surgical operation[3], extended burn injury[4], multiple trauma[5], severe sepsis[6-11] or pregnancy[12]. It is important to investigate the alteration in energy metabolism of critically ill patients and to determine an appropriate energy source and its dose for metabolic management.

The amount of oxidation of energy substrates; glucose or fat, has been assessed by the indirect calorimetry. Indirect calorimetry, however, is of limited use in measuring energy substrates utilized actually, since it cannot differentiate between direct oxidation of glucose and conversion of an equal amount of fat along with oxidation of the same amount of fat.

Direct measurement of the utilization of energy substrates has also been made using isotopes. The measurement of glucose oxidation using the primed constant infusion of [U-^{13}C]glucose was made in patients who underwent surgery for esophageal cancer, assuming highly stressed operative procedure, preoperatively and on the 3rd postoperative day. The results obtained from isotopic measurement and those from indirect calorimetry were discussed.

II. Materials and Methods

Subjects

Four patients who underwent operation for esophageal cancer, resection of thoracic esophagus followed by reconstruction using the gastric tube, including three fields lymph nodes dissection, were studied. They had neither diabetes meritus nor hepatic or renal dysfunction. They were all fed exclusively by total parenteral nutritiion (TPN) from at least 7 days before operation until 7 postoperative day. Characteristics of patients, together with the plasma levels of glucose, free fatty acid (FFA) and ketones are shown in Table 1. Plasma glucose, FFA and ketones were determined by enzymatic method. Informed consent was obtained prior to conducting these studies, as approved by the Research Ethics Committee of Chiba University School of Medicine.

Materials

D-[U-^{13}C] glucose (99.5 atom%^{13}C) and $NaH^{13}CO_3$ (99.9atom%^{13}C) were purchased from Chlorella Industry Co., Ltd., Tokyo, Japan. Isotope solutions were prepared as sterile and pyrogen-free conditions in normal

Table 1. Characteristics and Plasma Glucose, FFA and Ketones of Four Patients with Esophageal Carcinoma Treated by Esophagectomy

patient	age	gender	b.w.	Glucose mg/dl		FFA mEq/l		Ketones μmol/l	
				"pre"	"3pod"	"pre"	"3pod"	"pre"	"3pod"
1.	57	male	62	131	194	0.04	0.09	36	51
2.	50	male	54	133	237	0.02	0.14	40	64
3.	51	female	51	156	196	0.01	0.19	42	59
4.	58	male	50	107	153	0.06	0.09	40	52

"pre": preoperative, "3pod": third postoperative day

saline at the pharmacy department of Chiba University Medical Center.

Isotope Infusion Procedure

The details of the isotopic measurement of glucose oxidation will be published elsewhere. Briefly, blood and expired air samples were collected before infusion of the isotopic tracers at 9 a. m., for determination of the basal, natural abundance level of isotopic enrichment in plasma ^{13}C-glucose and in expired air for $^{13}CO_2$. Energy expenditure and CO_2 production were also determined by indirect carolimetry (Metabolic Gas Monitor mgm/two, Utah Medical, USA). Priming doses of $NaH^{13}CO_3$ at a dosage of 0.32mg/kg and D-[U-^{13}C]glucose at a dosage of 0.32mg/kg were then injected. Continuous infusion of D-[U-^{13}C] glucose was begun and continued for 130 minutes, using a syringe pump (ATOM pump, ATOM co. Ltd., Tokyo, Japan), at an infusion rate of 0.004mg/kg/min. Then blood and expired air were collected at 110, 120 and 130 minutes following the initiation of infusion for the measurement of isotopic enrichment. Indirect calorimetry was also performed each time. Amount of glucose, fat (tripalmitin) and protein metabolized were calculated by indirect calorimetry using the ordinary equations [13]. All these procedures were made during TPN providing 35 kcal/kg/day and 1.5g of protein/kg/day.

Analytical Methods

Blood samples. All blood samples were immediately centrifuged at 4℃, and the plasma was kept frozen at −20℃ until used for analysis. For determination of isotopic enrichment in plasma glucose, the glucose was isolated by precipitating plasma proteins and then passing the supernatant through anion and cation exchange columns (Dowex 50 W-X8, Amberlite IR-45, St. Louis, U. S. A.) according to Exton and Park[14]. The resultant elute was conbusted in a vacuum oven at 800℃ and the resulting CO_2 was analyzed for ^{13}C enrichment with the aid of a standard dual collector isotope ratio mass spectrometer (Delta E model, Finnigan MAT GmbH, Bremen, Germany).

Expired air. Expired air was collected for five minutes periods in a 100L Douglas Bag, and was transferred to an evacuated tube. Expired CO_2 was also analyzed for ^{13}C enrichment with the aid of an isotope ratio mass spectrometer (Delta E model, Finnigan MAT GmbH, Bremen, Germany).

Calculation of Glucose Kinetics by Isotopic Measurement

The glucose oxidation is calculated by the following equation[15]:

Percent of CO_2 due to glucose oxidation (%) $= (Eco_2/0.81)/Ep$ (1)

VCO_2 due to glucose oxidation (l/kg/day) $= (Eco_2/Ep) \times (Vco_2/0.81)$ (2)

Glucose oxidation (kg/kg/day) $= (Eco_2/Ep) \times (Vco_2/0.81)/0.746$ (3)

, where Eco_2 is the plateau $^{13}CO_2$ enrichment in the expired air, Ep is the plateau ^{13}C enrichment in plasma glucose, VCO_2 is liters of CO_2 expired per kg per day, 0.746 is liters of CO_2 from oxidation of 1g of glucose[15] and 0.81 is the estimation fraction of CO_2 recovered in expired air[16].

Plateau enrichments of ^{13}C of plasma glucose and expired air were obtained as the mean values of those of 110, 120 and 130 minutes after the initiation of infusion. Protein oxidation was calculated from the nitrogen loss into the urine. Production of CO_2 from fat oxidation was calculated by subtracting the CO_2 production due to the oxidation of glucose and protein from the total CO_2 production.

Ⅲ. Results

Plasma levels of FFA and ketone bodies increased on the 3rd postoperative day (Table 1). The increases appeared due to the increase of lipid mobilization and oxidation in the highly stressed state following operation for esophageal carcinoma (Table 1).

The results from indirect calorimetry are shown in Table 2. Levels of energy expenditure as well as O_2 consumption and CO_2 production increased in all of the patients on the 3rd postoperative day when compared to preoperative values. Production of CO_2, due to the oxidation of protein, elevated with the increased postoperative protein catabolism after operation. Amount of glucose oxidize, calculated from indirect calorimetry, increased in three patients out of the four. Nonprotein RQ was more than 1.0 in three patients, indicating more synthesis of fat rather than oxidation in the severely stressed state after esophagectomy. Amount of fat synthesis increased in two and fat oxidation turned to synthesis in one patient postoperatively.

Data of isotopic measurement are shown in Table 3. Production of CO_2 due to glucose oxidation and the amount of glucose oxidized

Table 2. Data from Indirect Calorimetry

		VCO_2	VO_2	EE	Urinary Nitrogen	npRQ	Glucose Oxidation	Net Fat Oxidation/Synthesis
		l/kg/d	l/kg/d	kcal/k/d	l/kg/d		g/kg/d	g/kg/d
1.	pre	5.19	5.23	26.0	8.90	1.045	6.32	− / 0.33
	3 pod	7.16	6.23	32.4	10.46	1.224	11.99	− / 2.01
2.	pre	4.97	4.93	24.8	6.48	1.035	6.47	− / 0.26
	3 pod	7.23	7.16	35.9	10.59	1.043	9.41	− / 0.45
3.	pre	6.48	6.29	31.8	6.45	1.059	8.98	− / 0.56
	3 pod	6.29	6.71	32.9	12.82	0.972	6.40	0.25 / −
4.	pre	4.65	5.06	24.7	6.46	0.936	4.51	0.46 / −
	3 pod	6.48	6.61	32.8	12.78	1.031	7.56	− / 0.29

"pre": preoperative, "3pod": third postoperative day
EE, energy expenditure ; npRQ, nonprotein Quotient Net fat oxidation or synthesis were calculated by different equation when npRQ was below or above 1.0, respectively.

Table 3. Data from Isotopic Measurement using primed constant infusion of [U-^{13}C]glucose

Patient		Protein			Glucose			Fat		
		Co_2 from P.oxid l/kg/d	%Co_2 from P.oxid %	Protein oxidized g/kg/d	Co_2 from G.oxid l/kg/d	%Co_2 from G.oxid %	Glucose oxidized g/kg/d	Co_2 from F.oxid l/kg/d	%Co_2 from F.oxid %	Fat oxidized g/kg/d
1.	pre	0.92	17.8	1.18	3.29	63.4	4.41	0.98	18.8	0.69
	3 pod	1.09	15.2	1.02	4.06	56.8	5.45	2.01	28.1	1.41
2.	pre	0.51	10.3	0.65	3.29	66.2	4.41	1.17	23.5	0.82
	3 pod	0.83	11.5	1.60	3.74	51.8	5.02	2.65	36.7	1.86
3.	pre	0.58	9.0	0.75	5.19	80.1	6.96	0.71	10.9	0.50
	3 pod	1.16	18.4	1.48	3.94	62.6	5.28	1.20	19.0	0.84
4.	pre	0.62	13.3	0.79	2.39	51.4	3.20	1.64	35.3	1.15
	3 pod	1.22	18.9	1.59	2.23	34.3	2.98	3.03	46.8	2.13

"pre": preoperative, "3pod": third postoperative day

were increased in two patients and decreased in two. Contribution of CO_2 production (% CO_2 production) due to glucose oxidation to total CO_2 production, however, decreased in all of the patients on the 3rd postoperative day. On the other hand, levels of both CO_2 production and % CO_2 production due to fat oxidation, and the amount of lipid oxidized increased in all of the patients postoperatively when compared to the preoperative values.

IV. Discussion

A methodology for the measurement of glucose oxidation using stable isotopes in humans has been established[12]. Primed constant infusion method is one of the most simple and convenient methods to use for surgical patients, because they are usually fed by TPN providing glucose by constant in fusion. Even though glucose metabolism alters significantly in the critically ill state and a relatively large amount of glucose is administered by TPN, the applicability of this method was confirmed in this study.

Energy metabolism in critically ill patients is not thoroughly understood. It is still controversial as to which of the energy subs trate, glucose, fat or others, is most effectively utilized in the critically ill patients. As most of the patients are managed by total parenteral nutrition, a relatively large amount of administered glucose makes the investigation of energy metabolism more complicated.

We have observed the increased plasma levels of FFA and ketones in the patient group who underwent operation for gastric or colorectal carcinomas, assuming moderately stressed operation, when the patients did not receive TPN. We have also observed that postoperative elevation of FFA and ketones in moderately stressed patients was completely suppressed by perioperative TPN[17].

In the present study, however, FFA and ketones increased in all four patients who underwent operation for esophageal carcinoma in spite of perioperative management by TPN. It was suggested that the mobilization and oxidation of fat were not suppressed by the administration of TPN in the highly stressed state such as that following esopha gectomy.

These observations match closely to the reports by other investigators. Glucose intolerance was reported to reflect their underlying severity of illness[5]. Hyperglycemic glucose clamp and forearm glucose uptake study revealed impaired glucose utilization in septic patients[9]. Askanazi *et al.* reported that septic and injured patients seem to preferentially utilize endogeneous fat as an energy source from the data obtained by indirect calorimetry[10]. In the present study, however, it was also observed that the nonprotein RQ exceeded 1.0 after operation in three patients out of four, indicating that fat was being synthesized rather than oxidized in the highly stressed state. Furthermore, glucose oxidation calculated from indirect calorimetry increased in three patients out of four.

Askanazi *et al.* also reported an RQ significantly less than 1.0 despite the fact that the patients received glucose in quantities to meet measured resting energy expenditure[10]. Stoner *et al.* published similar results with septic patients using RQ values to delineate fuel oxidation[11]. Although the data are in teresting, it is possible that not enough glucose was provided and low RQ values by definition reflect decreased glucose oxidation. It was revealed, in an other report, that RQ exceeded 1.0 by increasing the infusion rate of glucose even in severely burned patients[4]. Recently, it was reported that net fat synthesis was found as RQs exceeded 1.0 in 47% of ICU patients receiving TPN and mechanical

ventilatory support[3]. Thus, indirect calorimetry and RQ values are not reliable to clarify, which of the fuel substrates are preferentially utilized in the critically ill patients.

Isotopic measurement has been used to further investigate the metabolism of energy fuels in critically ill patients[12]. According to the findings from the present study, $\%CO_2$ production from glucose oxidation decreased in all of the patients. On the other hand, both CO_2 production and $\%CO_2$ production due to fat oxidation and the amount of lipid oxidized increased in all of the patients post operatively, when compared to the preoperative values. These findings closely corresponded to the increased FFA and ketones after operation.

Indirect calorimetry measures net fat oxidation (nonprotein RQ below 1.0) or net lipogenesis (nonprotein RQ above 1.0), but both oxidation and synthesis of lipid may occur simultaneously. Lipogenesis in some tissues may be appreciable when the RQ is below 1.0, if fat oxidation occurring elsewhere is greater. In the present study, it is strongly suggested that fat oxidation increased together with the simultaneous and greater increase of lipogenesis from glucose in the highly stressed state such as postesop hagectomy. These important findings can be obtained from the combination of both isotopic and indirect calorimetric measurements.

Energy metabolism in the stressed state or other pathophysiological states will be clarified by the investigation using the combination of both isotopic and indirect calorimetric measurements.

Appropriate energy substrate and its dose which is effectively utilized in the pathophysiological state will be determined applying this technique on a larger number of patients.

要　旨

重症患者では糖，脂肪，蛋白などの代謝回転が亢進しているが，大量のグルコースが投与されて栄養管理が行われているため，エネルギー代謝を間接熱量測定だけで把握することは困難と考えられる。我々は，間接熱量測定と安定同位元素を用いたエネルギー代謝動態の測定法とを併用し，間接熱量測定の限界および安定同位元素による測定法の有用性を検討した。

三領域リンパ節郭清を伴う右開胸開腹胸部食道全摘再建術を施行し，高カロリー輸液（TPN）管理した食道癌患者4例について，術前と術後3病日に，グルコース酸化速度を [U-^{13}C]glucose による primed constant infusion 法により測定し，蛋白酸化量を尿中窒素排泄量から，脂肪酸化量を総 CO_2 産生量からグルコース及び蛋白の酸化による CO_2 産生量を除いて算出した。同時に間接熱量測定を施行した。また，エネルギー代謝の指標として血糖，血中 FFA，及びケトン体のそれぞれの値を測定した。

血中 FFA とケトン体値は TPN 管理にもかかわらず術後全例で上昇した。従って脂肪の動員と酸化亢進が示唆された。間接熱量測定では，グルコースの酸化量が3例で増加しており，非蛋白呼吸商（npRQ）が3例で1.0以上を示し，脂肪は酸化よりも合成に傾いていた。しかし，安定同位元素による測定では，脂肪の酸化は全例で増加しており，グルコースの酸化は全例で抑制された。間接熱量測定では，脂肪が酸化されていても等量の脂肪がグルコースから合成されるとグルコースの酸化として算出されるためと思われた。安定同位元素を用いた測定法では，重症患者においてグルコースの酸化抑制と脂肪の酸化亢進があること，脂肪は酸化されている以上に合成され代謝回転が亢進していることが示唆された。

以上，重症患者のエネルギー代謝の検討には，間接熱量測定法に安定同位元素法を併用することが必須であると考えられた。

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