EVALUATION OF TUBE FEEDING FORMULAS ON MICROBIOLOGICAL SAFETY AND PROTEIN METABOLISM IN PATIENTS WITH CARCINOMA OF THE LARYNX

PANWADEE PUTWATANA

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ชื่อวิทยานิพนธ์ การประเมินผลการให้สูตรอาหารทางสายให้อาหารต่อความปลอดภัยด้านจุลินทรีย์และเมตาบอลิซึมของโปรตีนในผู้ป่วยโรคมะเร็งกล่องเสียง

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บทคัดย่อ

การขาดโปรตีนและพลังงานเป็นปัญหาสำคัญของผู้ป่วยในโรงพยาบาล โดยเฉพาะผู้ป่วยโรคมะเร็ง การศึกษานี้ เพื่อประเมินผลของ สูตรอาหาร ที่ให้ทาง สายให้อาหาร 2 สูตร คือ Isocal RTU ซึ่งเป็น สูตรอาหารสำเร็จรูป กับ Ramathibodi blenderized formula ซึ่งเป็นสูตรอาหารที่เตรียมโดยโภชนากรของโรงพยาบาลรามาธิบดี ต่อความปลอดภัย ด้านจุลินทรีย์ และเมตาบอลิซึม ของโปรตีนในผู้ป่วยมะเร็ง กล่องเสียง จำนวน 60 คน แบ่งเป็น 2 กลุ่ม กลุ่มที่ 1 40 คน ได้รับ Isocal RTU และกลุ่มที่ 2 20 คน ได้รับ RBF ทางสายให้อาหาร วันผ่าตัดและวันที่ 1 หลังผ่าตัด ผู้ป่วยได้รับอาหารเพียงบางส่วน ทางหลอด เลือดดำ ตั้งแต่วันที่ 2 หลังผ่าตัด ได้รับสูตรอาหารทางสายให้อาหาร โดยเฉลี่ยต่อวันกลุ่ม 1 ได้รับพลังงาน 1,808 กิโลแคลอรี่ เป็นโปรตีน 58 กรัม ไขมัน 76 กรัม มอลโตเด็กซ์ตริน (Maltodextrin) 228 กรัม ส่วนกลุ่ม 2 ได้รับพลังงาน 1,902 กิโลแคลอรี่ เป็นโปรตีน 75 กรัม ไขมัน 67 กรัม และซูโครส 234 กรัม การศึกษาพบว่า ผู้ป่วยกลุ่ม 1 และ 2 ไม่มีความแตกต่างกันในเรื่อง อายุ เพศ โรคที่เป็น และการผ่าตัด โดยมีค่ามัชฌิม $\pm$ ค่าความคลาดเคลื่อนมาตรฐาน ของอายุ 62.85 ± 1.41 และ 60.25 ± 2.51ปีตามลำดับ ผู้ป่วยทั้ง 2 กลุ่มมี ภาวะขาดโปรตีน และพลังงานในระดับเดียวกัน ผลจาก

การผ่าตัดและการได้รับ อาหาร เพียงบางส่วน ทางหลอดเลือดดำ เป็นเวลา 2 วัน ทำให้ภาวะขาดโปรตีน และพลังงานเพิ่มขึ้น โดยมีค่าของสัดส่วนต่างๆของร่างกาย ลดลง มีค่าโปรตีนต่างๆในเลือดลดลงอย่างมีนัยสำคัญทางสถิติ ได้แก่ ค่าโปรตีนทั้งหมด แอลบูมิน ทรานส์เฟอร์ริน อาร์บีพี อะโป เอ-I และบี ยูเรียไนโตรเจน เอนไซม์ เอ.แอล. ที. และ จี.จี.ที. นอกจากนี้ค่าฮีโมโกลบิน ฮีมาโตคริท และเม็ดเลือดแดง ก็ลดลงด้วย เมื่อได้รับอาหารทางสายให้อาหาร เป็นระยะ 3-11 วัน พบว่าเพียงพอที่ทำให้เกิดผลดี ต่อภาวะโปรตีนและพลังงาน โดยส่วน ที่เป็นไขมัน และไม่ใช่ไขมันในร่างกายไม่ลดลงอีก ดุลไนโตรเจนเพิ่มขึ้น จนเกือบเป็นดุลบวก ค่าโปรตีนต่างๆ ในเลือด เพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ได้แก่ โปรตีนทั้งหมด แอลบูมิน ทรานส์เฟอร์ริน อาร์บีพี อะโปเอ-I และบี ยูเรียไนโตรเจน และครีเอตินีนคาร์นิทีน ในปัสสาวะเพิ่มขึ้น ในวันแรกหลังผ่าตัดนั้น เป็นผลมาจาก ร่างกายตอบสนองต่อความเครียดจากการ ผ่าตัด แม้ว่าค่าคาร์นีทีนในปัสสาวะ ลดลงในช่วงวันที่ 2-12 ภายหลังการผ่าตัด แต่ผู้ป่วยทั้ง2กลุ่ม มีดุลคาร์นิทีนเป็นลบ เพราะได้คาร์นิทีน จากสูตรอาหาร ไม่เพียงพอ

การศึกษาด้านความปลอดภัยด้านจุลินทรีย์ในสูตรอาหาร และชุดให้อาหารทางสาย ได้ประเมินโดยการนับจำนวนแบคทีเรีย และแยกชนิดของเชื้อ โดยแบ่งผู้ป่วยกลุ่ม 1 ที่ได้รับ Isocal RTU ออกเป็นกลุ่มละ 20 คน เพื่อให้อาหารโดยวิธีหยอดอย่างต่อเนื่อง ในระบบปิดและระบบกึ่งปิด กลุ่ม 2 ที่ได้สูตรอาหาร RBFโดยแขวน ให้หยดเป็นมื้อด้วยชุดให้อาหาร ที่ดัดแปลงจากชุดให้สารน้ำทางหลอดเลือดดำที่ใช้แล้วมาล้างทำความสะอาด พบว่า สูตร อาหาร Isocal RTU มีความปลอดภัยด้านจุลินทรีย์ แต่เมื่อแขวนให้แก่ผู้ป่วย 12 ชั่วโมง ด้วยระบบปิดหรือระบบกึ่งปิด พบว่ามีอัตราการปนเปื้อนเชื้อเพิ่มขึ้น ส่วนสูตรอาหาร RBF พบว่ามีการปนเปื้อนเชื้อตั้งแต่ก่อนเริ่มให้แก่ผู้ป่วย เมื่อแขวนให้แก่ผู้ป่วย 1 ถึง 1 1/2 ชั่วโมง มีการปนเปื้อนเชื้อทั้งหมด นอกจากนี้ พบว่า มีการปนเปื้อนเชื้อที่ชุดให้อาหารด้วย เชื้อที่พบทั้งหมด เป็นเชื้อที่พบ เป็นประจำ ในโรงพยาบาลรามาธิบดี

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ABSTRACT

Protein-energy malnutrition is an important problem of hospitalized patients especially in cancer patients. The purpose of this study is to evaluate 2 types of enteral formulas, ie. Isocal RTU (a commercial one) and Ramathibodi blenderized formula (RBF: a dietetic-prepared formula) on their microbiological safety and protein metabolism in patients with carcinoma of the larynx. Sixty patients were divided into 2 groups, ie, groups I and II consisted of 40 and 20 patients receiving Isocal RTU and RBF, respectively. Patients in both groups were rather homogeneous in terms of, age, gender, underlying diseases, and types of operation. Their mean(±SEM) age in groups I and II were 62.85±1.41 and 60.25±2.51 yrs, respectively. Both groups of patients had inadequate protein-energy status and the severity of protein-energy disorder was in the same order. On the day of operation (D0) and the first postoperative day (D1), both groups recieved only partial parenteral nutrition derived from glucose only. Starting from D2 they were tube-fed for 3-11 days (D5-13 postoperatively). The mean daily energy, protein, fat, and carbohydrate intakes in groups I were 1,808 kcal, 58, 76, and 228 g, respectively,

whereas the corresponding figures in group II were 1,902 kcal, 75, 67, and 234 g . The results show that surgical stress and inadequate nutrition support during D0-1 caused the deterioration of their protein-energy status at D2 evidenced by the decreases in their body composition parameters and serum total protein, albumin, transferrin, RBP, apo A-I, apo B, urea nitrogen, ALT and GGT levels. In addition, there were decreases in Hb, Hct, and RBC counts. After tube fedings for 3-11 days, the beneficial effects of Isocal RTU or RBF on protein-energy status were shown by no further significant decreases in their body fat and fat-free mass, significantly increases in their serum total protein, albumin, transferrin, RBP, apo A-I, apo B, urea nitrogen and creatinine levels and their nitrogen balances were markedly less negative. The significant increases in urinary total carnitine excretion at D1 were due to surgical stress stimuli. Both groups had inadequate carnitine intakes during recieving enteral formulas that lead to negative carnitine balances.

Microbiological safety of enteral formulas and feeding sets were assessed. The first 20 patients on Isocal RTU were fed by continuously closed system, the second 20 patients on Isocal RTU by continuously semi-closed system, whereas the remaining 20 patients on RBF were fed by opened intermittent system. Bacterial contamination was determined by pour plate method with dilution before and at the end of each feeding period. Isocal RTU in Tetrapak was sterile. After it was hung for 12 hrs, in the closed or semi-closed system, it was bacterial contaminated. RBF was bacterial contaminated prior to the administration and all were contaminated at the end of 1 to 1 1/2 -hr feeding. The feeding set was also bacterial contaminated. All bacteria identified in this study are commonly found at Ramathibodi Hospital.

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LIST OF ABBREVIATIONS

ALT	Alanine aminotransferase
AP	Alkaline phosphatase
Apo A	Apoprotein A
Apo B	Apoprotein B
AST	Aspartate aminotransferase
BFM	Body fat mass
BFMI	Body fat mass index
BMI	Body mass index
Ccr	Creatinine clearance
cm	Centimeter
CK	Creatine kinase
D	Day
dl	Deciliter
EDTA	Ethylene diamine tetraacetic acid
F	Female
FBG	Fasting blood glucose
FFMI	Fat free mass index
g	Gram
GGT	Gamma glutamyl transferase
Hb	Hemoglobin
Hct	Hematocrit
HD	Habitual diet
HDL	High density lipoprotein
HMG-CoA	3-Hydroxy 3-methyl glutaryl CoA
hr.	hour

i.e.	id est (that is)
kcal	Kilocalorie
IV	Intravenous
kg	Kilogram
L	Lymphocyte
M(Sex)	Male
M	Monocyte
m	meter
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
mcg	microgram
mg	milligram
min	minute
mL	milliliter
mOsm	milliosmolar
MUAC	Mid upper arm circumference
MUFA	Monounsaturated fatty acid
N	Neutrophil
NPC:N	Non-protein-calorie:nitrogen
OV	Ovovegetarian
PCM	Protein-calorie malnutrition
Pg	Picogram
PF	Protein free
PL	Phospholipid
PPN	Partial parenteral nutrition
RBC	Red blood cell
RBF	Ramathibodi blenderized formula

RBP	Retinol-binding protein
RD	Regular diet
RE	Retinol equivalent
RTU	Ready-to-use
Scr	Serum creatinine
Std	Standard
SEM	Standard error of mean
TBW	Total body water
TE	Tocopheral equivalent
TST	Triceps skinfold thickness
UAMC	Upper arm muscle circumference
Ucr	Urinary creatinine
uM	micromole
V	urinary volume
VD	Vegetarian diet
VLDL	Very low density lipoprotein
WBC	White blood cell
WHR	Waist-over-hip circumference ratio
wk	week
yr	year

LIST OF FIGURE

LIST OF TABLES

CHAPTER I

STATEMENT OF THE PROBLEMS AND OBJECTIVES

Even the advance knowledge in the science of nutrition and food technology nowadays, protein-calorie malnutrition (PCM) is still a major problem of the various populations in many parts of the world especially in the developing countries including Thailand[1,2]. Furthermore, PCM is also the most common nutritional problem found in hospitalized patients[2,3]. The prevalence of PCM based on weight for height in 140 medical patients and 67 surgical patients at Ramathibodi hospital were 73 and 79% respectively[4].

Cancer patients have the highest incidence of PCM in hospitalized patients[5]. Many patients are malnourished at the time of diagnosis. To this already compromised individual, the stress of treatment is added. All of three major treatment modalities of surgery, radiation therapy, and chemotherapy have deliterious nutritional consequences[6,7]. For instance, the surgical treatment compounds the metabolic derangements and further increases the risk of postoperative morbidity and death. The nutritional effects of cancer surgery are a result of general metabolic response to surgery in acute phase and the partial or complete resection of specific organs in the chronic phase[5-7].

Cancer of the head and neck including the lips, the oral cavity, the pharynx and the larynx is in ten leading site of cancers in both male and female adulthood[8]. The potential nutritional consequences in those patients are mechanical difficulty associated with food ingestion and altered capability to ingest food. During the immediate postoperative period, they must depend on tube feeding for their nourishment.

Enteral formula can be provided through various sites of gastrointestinal tract, depending on the clinical condition of the patients. The most common route is nasogastric tube which is convenient and low cost.[9] Several enteral formulas are available commercially and the use are growing[10,11]. However, enteral formulas prepared by dietitians in the hospitals are still commonly employed in Thailand. Such formulas are freshly prepared from various food items for daily use. In general the commercial formulas are more perfect in terms of the balance of macronutrients, micronutrients as well as some important physico-chemical properties while the dietitian-prepared formulas are on the level of macronutrients. This difference may affect the effectiveness of the feeding and the absorption of nutrients.[12] However, most of the commercial formulas available in Thailand are imported. So their prices are rather high and this factor limits the prescription. Though there is a report to compare the price, composition, and other properties of the commonly used commercial formulas and dietitian-prepared formulas[13], the evaluation based on clinical practice and outcome has never been performed.

Statement of the problems

Nutritionally complete enteral formulas are not only nutritious for patients' survival but also for the growth of contaminated microorganisms which is liable to occure[14-16]. Thus tube feeding is a potential source of infection which is particularly dangerous to the debilitated or compromised patients. The high frequency of microbial contamination of enteral nutrition solutions was well established since 1975[17]. However, this problem has never been studied in Thailand which is located in the tropical area and prone to develop microbial contamination.

Objectives

The present study was therefore carried out to evaluate 2 types of enteral formulas, ie, a common commercial one and a dietetic-prepared formula on their microbiological safety and protein metabolism in patients with carcinoma of the larynx.

The specific aims of the study are to evaluate the microbiological safety and protein metabolism in 2 groups of patients with carcinoma of the larynx who were tube fed during the postoperative period by Isocal ready-to-use (RTU) or Ramathobodi blenderized formula (RBF).

The microbiological safety was based on the results of the number and types of bacterial contamination in the aforesaid 2 enteral formulas which were administered by 3 methods, ie, continuously closed system, continuously semi-closed system for the Isocal RTU and intermittently opened system for the RBF before and at the end of each feeding period.

Protein metabolism of the patients was assessed at 3 intervals during the hospitalization, ie, preoperative period, postoperative period before enteral tube feeding, and postoperative period at the end of enteral tube feeding.

Parameters employed to evaluate this were dietary assessment, anthropometric measurement, and biochemical determination including nitrogen balance visceral protein status, carnitine, creatinine, and uric acid status.

CHAPTER II

MATERIALS AND METHODS

Patients

From January 1991 to March 1993, the study was conducted in 60 adult patients with cancer of the larynx who were admitted at the otolaryngology wards, Ramathibodi hospital, Bangkok. Thailand, for surgical treatment and planned to have enteral tube feeding during the postoperative period. Forty patients were fed with Isocal RTU and the other 20 patients were fed with RBF.

For the Isocal RTU group, the first 20 patients were fed by continuously closed system, the second 20 patients by continuously by semi-closed system, whereas the remaining 20 patients on RBF were fed by opened intermittent system. Only the patients who recieved enteral tube feeding for more than 4 days, were included in the study.

All subjects were inserted enteral tube during the preoperative or intraoperative period by the physicians by the standard procedure.

Enteral formulas :

Isocal RTU[18]

Isocal is a nutritionally complete and lactose free enteral formula. Its ingredients consist of water, maltodextrin, soy oil, calcuim caseinate, sodium caseinate, medium chain triglycerides (fractionated coconut oil), soy protein isolate, potassium citrate, lecithin, calcium citrate, magnesium chloride, calcium chloride, dibasic calcium phosphate, dibasic magnesium phosphate, sodium citrate, carrageenan, potassium chloride, vitamins and minerals with osmolarity of 300 mOSm/kg H_2O. The nutritive values are presented in **Table 1-5.** The ready-to-use in the package (Tetrapak) is the new product in Thailand that is claimed to be more convenient, hygeinic and microbiological safe by using the corresponded feeding set. It is imported by Mead-Johnson Nutritionals, Bristol-Myers Squibb (Thailand) Ltd.

Ramathibodi Blenderized Formula (RBF)

RBF is prepared daily by the staff of the Division of Nutrition and Dietetics. It contains the following ingredients[19] : 100 g of, hen liver, pumpkin, banana and sucrose ; 200 g of hen egg ; 10 g of vegetable oil and water. Its osmolarity is 614 mOSm/kg H_2O. RBF is usually prepared at the energy content of 1 kcal/mL. Its nutritive values of the formula are presented in **Tables 1,2,4, and 5.**

Delivery systems :

The continuously closed system.

The system sonsisted of the liquid form of Isocal RTU in a closed, sealed, 970 mL package ; the Iso-Flo Spike which is the dispossiable enteral tube-feeding set with the spike to puncture to the formula package and it has a roller clamp for adjusting the flow rate and a Luer Lok for adding liquid to the feeding (the extension tubing was changed every 24 hr.) ; and the spring scale for hanging the formula package to a stand and reflexing the remaining volume of the formula inside the package during feeding.

The continuous semi-closed system

The system consisted of Dobbhoff enteral feeding bag which is a dispossaible 1,000 mL feeding bag with feeding set. The reservoir bag and extension tubing were changed every 24 hr.

The intermittent opened system

This system is generally used in Ramathibodi hospital inorder to prolong the duration of feeding more than the bolus method by syringe feed as used in the prior period.

The system consisted of plastic bottle and delivery set which had already been used for intravenous infusion and cleaned. The daily feedings were divided equally into 6 times at 8.00, 11.00, 14.00, 17.00, 20.00, and 23.00 o' clock. Steamed RBF in glass bottles were delivered from the Division of Dietetics to wards and kept in the refrigerator at 4°c until the administration to the patients at the aforesaid schedule. The glass bottle containing RBF was removed from the refrigerator and stand at room temperature for 30 mins. Then RBF was transfered from the glass bottle to the plastic bottle through its bottom which was cut-off. The formula was administered by gravity drip method for 2 hrs feeding through the delivery set connecting between the plastic bottle and the enteral tube.

Table 1. Macronutrient, ash, and moisture content of Isocal RTU and RBF

Composition	Formula	
	Isocal RTU[18]	RBF[13]
	< -------- per 100 kcal ---------->	
Protein (g)	3.2	4.08
Fat (g)	4.2	3.64
Carbohydrate (g)	12.6	12.73
Ash (g)	0.6	0.48
Moisture (g)	84.5	75.08

Table 2. Energy content, energy distribution and non protein calorie : nitrogen ratio of Isocal RTU and RBF

Composition	Isocal RTU[18]	RBF[13]
Protein (%)	16.32	12.8
Fat (%)	32.75	37.8
Carbohydrate (%)	50.93	50.4
NPC : N*	167:1	128.1
Energy (kcal/mL)	1	1.08

*Non-protein-calorie : nitrogen

Table 3. Amino acid composition of Isocal RTU[18]

Amino acid	Amount
	mg/100 kcal
Essential	
Histidine	86
Lysine	230
Tryptophan	38
Phenylalanine	160
Tyrosine	150
Methionine	77
Cystine	16
Threonine	128
Leucine	310
Isoleucine	170
Valine	220
Nonessential	
Arginine	122
Alanine	106
Aspartic acid	270
Glutamic acid	700
Glycine	77
Proline	320
Scrine	160

Table 4. Vitamin contents of Isocal RTU and RBF

Vitamin		Isocal RTU[18]	RBF[13]
		< -------- *per 100 kcal* ---------->	
A	(IU)	250	304.65gRE
B1	(mcg)	192	48
B2	(mcg)	168	163.5
B6	(mcg)	250	211.5
B12	(mcg)	0.75	441,500
C	(mg)	15	-
D	(IU)	20	-
E	(IU)	3.8	0.84mgTE
Pantothernic acid (mcg)		1250	845
Niacin	(mcg)	2500	710.5
Folic acid	(mcg)	20	144
Biotin	(mcg)	15	-
K	(mcg)	12.5	-
Choline	(mcg)	25	-

- not determined

Table 5. Mineral contents of Isocal RTU and RBF

Mineral		Formulas	
		Isocal RTU[18]	RBF*
		< -------- *per 100 kcal* ---------->	
Calcium	(mg)	60	17.17
Phosphorus	(mg)	50	56.55
Magnesium	(mg)	20	7.27
Sodium	(mg)	50	70.89
Potassium	(mg)	125	94.51
Chloride	(mg)	100	44.17
Iron	(mg)	0.9	1.15
Zinc	(mg)	1.0	0.65
Copper	(mg)	0.1	0.08
Manganese	(mg)	0.3	-
Iodine	(mg)	7,500	-

* calculated from (13)

- not determined

Experimental design and treatment

On the operative day, the patients recieved only IV fluid from the operative room (OR) as prescribed by the surgeon. For the first (D1) and second postoperative days (D2), each patient recieved partial parenteral nutrition providing 1,000 and 600 kcal, respectively. The energy sources for PPN were derived from glucose only. The enteral tube feeding was started on the second postoperative day with either 970 kcal Isocal RTU or 960 kcal RBF. From the third day (D3) onwards, the patients recieved a daily supply of either 1,940 kcal Isocal RTU or 1,920 kcal RBF (**Table 6).**

Table 6. Schedule of feedings

Day	Isocal RTU	RBF
0	<---------- IV fluid ---------->	
1	PPN 1,000 kcal, 10 mEq KCl, 1 mL Pancebrin	
2	PPN 600 kcal, 10 mEq KCl, 1 mL Pancebrin	
	Isocal RTU* 970 kcal	RBF@ 960 kcal
	Total energy intake 1,570 kcal	1,560 kcal
3 onwards		
	Isocal RTU# 1,940 kcal	RBF$ 1,920 kcal

* 1 bottle of Isocal RTU/day ; rate : 40 mL/hr.

@ 2 bottles of Isocal RTU/day ; rate : 80 mL/hr.

\# 240 mL of RBF x 4 feedings/day ; rate : 240 mL within 2 hrs.

$ 320 mL of RBF x 6 feedings/day ; rate : 320 mL within 2 hrs.

Nurses got acquainted with the first 2 tube feeding delivery system by the slide-tape presentation of the procedure and provision of opportunity for some nurses to practice prior to the study. Both Isocal RTU and RBF were administered to the patients by the gravity drip method and flow rate was adjusted by the height of suspended container and the roller clamp.

Study on microbiological safety

Colleclion of enteral formulas

Each enteral formula sample was collected aseptically by using dispossible syringe and needle. For Isocal RTU, the sample was drawn from the formula container immediately before starting and just before completing the feeding, from the feeding container to obtain the sample after it was hung at the patient's bed side for 16 hrs(performed at night time), and from the feeding container after it was hung for 12 hrs (performed at day time).

For RBF, it was collected 3 times a day, in the morning, afternoon, and evening before and at the end of each feeding in the same manner as the Isocal RTU. For each subject, these food collection samples were done for 1 or 2 days.

Microbiological exammination

The determination for the presence of microorganisms in the enteral formula samples were based on plate count with dilutions by using the pore plate method[20] of plate count agar medium (Difco) and incubated at 37°c for 48 hrs. These would determine the number of colony forming units (cfu) per ml of sample. whenever more than 10 cfu/mL were present, microorganisms were identified.

Dietary assessment

During the study, daily actual volume intake in each patient was recorded by nurses, using the fluid intake/output flow sheet and the special tube feeding flow sheet . Daily total energy, protein, fat, and carbohydrate intake in each patient were then calculated, using published references.[13,18,19] The mean daily intake of nutrient for each patient was derived from the sum of each nutrient intake divided by actual days of feeding.

The problems of feedings accounted by the patients were also noted by the nurses.

Anthropometric measurement

Anthropometric measurement was conducted at D0,D2, and the last day of feeding(D5-13) in each patient. Only height was measured at D0. Other anthropometric parameters included body weight, triceps skin fold thickness (TST), mid upper arm circumference (MUAC), waist and hip circumferences were measured by using the standard techniques.[21-23] The upper arm muscle circumferences (UAMC) was calculated from the formula: UAMC = MUAC - 3.1416. TST Standards of TST, MUAC, UAMC were taken from the standard source[21]. Standard of weight for height was based on Metropolitan Life Insurance Company[24]. Body mass index (BMI)[25] was derived from weight in kg divided by height in m^2. Waist -over-hip circumference ratio (WHR) was derived from waist circumference[23] divided by hip circumference. The body fat of each patient was measured, using the technique of near-infrared light interactance by the Futrex 5,000-A Boby Composition Analyzer (Futrex Inc, Gaithersburg, MD, USA)[26].

Urine collection

Twenty-four hr urine, samples were collected using toluene as preservative at the preoperative day (D-1), and from D1 to the last day of feeding. Urine of D2-12 were pooled and analyzed as one specimen. The samples were kept frozen until biochemical analysis was performed.

Blood collection

Venous blood was obtained from each subject after a 8-10 hrs fast at days 0, 2 and the last day of feeding (D5-13). Serum, plasma, and erythrocyte, and erythrocyte were seperated and kept at -20°c for biochemical determination.

Hematological determination

Hemoglobin (Hb), hematocrit (Hct), red blood cell (RBC) count, white blood cell (WBC) count, platelet count, RBC indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined by the H-1 hematology analyzer[27] (Technicon Instruments Corp, Tarrytow). Total lymphocyte count was derived from WBC count x% lymphocyte/100.

Liver, and renal function tests and serum mineral levels

Serum total protein, albumin, urea nitrogen, bilirubin, aspartate aminotransferase (SAST), alanine aminotransferase (SALT), alkali phosphatase (AP), creatinine, uric acid, and minerals levels were measured by SMA-12 at General Clinical Chemistry Laboratory, Ramathibodi Hospital.

Other biochemical parameters

Serum transferrin, apoprotein (apo) A-I, and apo B were measured by the immunochemical reaction with specific antibodies. The turbidity developing on mixing of serum and reagent (Turbiquant) was measured by Turbitimer (Behring, marburg, W., Germany)[28]. Serum retinol binding protein (RBP) was determined by single radial immunodiffusion technique, using LC-partigen (E-Behring, Marburg, Germany).[29] Serum and urinary carnitine levels were determined by enzymatic-colorimetric assay of McGarry and Foster.[30]

Urinary nitrogen excretion was determined by Kjeldahl principle using Kjeltec system (Tecator, Sweden)[31]. Nitrogen balance was calculated from nitrogen intake-urinary nitrogen-2[32].

Urinary creatinine was determined by Jaffe's method after deproteinization[33] and urinary uric acid by the enzymatic method of Klose.[34]

Calculation of creatinine, carnitine, and uric acid clearance was used the same principle. For instance, creatinine clearance (Ccr) in ml/min was calculated from[35].

Ccr = Ucr x V/Scr

Ucr = urinary creatinine concentration in mg/mL

Scr = serum creatinine concentration in mg/mL

V = urinary volume in mL/min

To adjust for the difference in body surface area among the patients, all clearance values were adjusted to the body surface area of $1.73/m^2$.

Statistical analysis

Statistical analysis was performed by the Statistical Package for the Social Sciences (SPSS)[36]. The data of each period were reported as mean (± SEM) values. Comparisons were made within each group and between the 2 groups at the corresponding periods using paired and unpaired t-test. Regression analysis was also computed for some parameters.

CHAPTER III
RESULTS

Patients' profile

Sixty patients were divided into 2 groups, ie, group I consisted of 40 patients recieving Isocal RTU whereas group II consisted of 20 patients recieving RBF. Group I was further divided equally into 2 subgroups, ie, group IA recieving tube feeding by the continuously closed-system, whereas group IB recieving tube feeding by the continuously semi-closed system. **Tables 7, 8, and 9** show the initial characteristics of patients in group IA, IB, and II, respectively. Group I consisted of 37 men and 3 women with the means (± SEM) ages of 62.85 ± 1.41 yrs ; out of 40 patients 39 were carcinoma of the larynx. Out of 20 patients in group II 19 were men; their mean (±SEM) age was 60.25±2.50 yrs; 17 of them were careinoma of the larynx. In group I, 38 recieved enteral feeding through nasogastric tube, 1 through gastrostomy, and 1 through jeiunostomy, whereas all 20 patients in group II recieved enteral feeding through nasogastric tube.

In group I, 1 patient received tube feeding for 3 days, 1 for 4 days, 2 for 5 days, 8 for 6 days, 9 for 7 days, 9 for 8 days, 5 for 9 days, 1 for 10 days and 4 for 11 days. In group II, 1 received tube feeding for 5 days, 1 for 6 days, 7 for 7 days, 9 for 8 days, and 2 for 9 days. Thus 90% of patients in group I and 95% in group II received tube feeding for >6 days. The reason for the termination of tube feeding for <5 days was due to the removal of the tube by accident.

Microbiological safety

The incidences of bacterial contamination in Isocal RTU are shown in **Tables 10 and 11** for group IA and **Tables 12 and 13** for group IB whereas **Table 14** show for those in group II.

Format of presentation of other data

Since groups IA and IB received the same amount and types of enteral formula, ie, Isocal RTU, and venepuncture was done under the same condition, the results obtained from groups IA and IB were pooled together and presented as a single group: group I.

The data on dietary assessment **(Table 15)** anthropometric parameters **(Tables 16-19)**, serum protein levels **(Tables 23-25)**, serum urea nitrogen and creatinine levels **(Table 28)**, serum uric acid and fasting blood glucose (FBG) **(Tables 29)**, urinary creatinine, and uric acid excretions and their clearances **(Table 31)**, urinary nitrogen excretion **(Table 32)**, nitrogen balance **(Table 33)**, plasma and urinary carnitine levels **(Tables 34-36)**, carnitine clearance **(Table 37)**, hematological status **(Table 38)**, serum bilirubin and enzyme levels **(Table 39)** and serum mineral levels **(Table 40)** are presented by the same format as follows:

1. each parameter is presented as mean±SEM;
2. number of patients was indicated in the title of the **Table** only when some patients were not assessed for that particular parameter;

3. for each parameter, statistical comparisons were made between various study periods within the same group by paired t-test as well as between groups I and II at the corresponding periods by unpaired t-test and the significant differences are shown in the footnoot of each **Table**: a and b indicating significant difference from Do and D2, respectively, within the same group whereas A indicating significant difference between groups I and II at the corresponding periods; and

4. the study was divided into 3 periods, ie, Do (before the operation), D2 (1 day after the operation, and D5-13 (3 to 11 days for tube feedings), they were grouped together for statistical analysis and the majority received tube feeding for >6 days (90% in group I and 95% in group II).

Tables 20-22 show the prevalences of underweight, acceptable weight, and obesity by BMI in groups I, and II at Do, D2, and D5-13, respectively. **Table 26** shows the prevalences of inadequate visceral protein status in group I and II.

Relationship between various parameters

The relationships between various parameters were analyzed by regression analysis. They were referred in the discussion for supporting the finding based on the coefficient correlation (r) and p values.

Table 7. Initial characteristics in group IA patients

Name	Sex	Age	Diagnosis	Operation
KOu	M	53	CA larynx	Total laryngectomy and right modified radical neck dissection
VSr	M	66	CA larynx	Total laryngectomy and left neck node picking
CJa	M	64	CA larynx	Total laryngectomy
SNu	M	63	CA pyriform	Total laryngectomy and left radical neck dissection
LDo	M	68	CA larynx	Total laryngectomy and right radical neck dissection
CMa	M	61	CA pyriform	Laryngopharyngectomy and gastric pull up with jejunostomy
KTh	M	72	CA pyriform	Total laryngectomy and right radical neck dissection
JJi	M	55	Transglottic CA	Total laryngectomy
SKa	M	66	Transglottic CA	Total laryngectomy
HSo	M	66	Transglottic CA	Total laryngectomy and node picking
HTe	M	47	CA pyriform	Total laryngectomy and preserve left lower lobe thyroid with slide widening esophagus and colla excision
HTu	M	68	CA larynx	Total laryngectomy
APa	F	60	Subglottic CA	Total laryngectomy
BAn	M	69	CA epiglottis	Total laryngectomy and right modified radical neck dissection
SAm	M	80	CA pyriform	Total laryngectomy and right radical neck dissection
STh	M	61	CA larynx	Total laryngectomy
BKl	M	53	CA larynx	Total laryngectomy
CJr	M	56	CA larynx	Total laryngectomy and right radical neck dissection
PCa	M	76	CA larynx	Total laryngectomy
NNg	M	48	CA pyriform	Total laryngectomy and bilateral modified radical neck dissection

Table 8. Initial characteristics in group IB patients

Name	Sex	Age	Diagnosis	Operation
YPu	M	73	CA pyriform acute cholecystitis left pleural effusion	Total laryngectomy and right modified radical neck dissection with left supraomohyoid neck dissection
CJe	M	64	CA larynx	Total laryngectomy and total thyroidectomy
SVo	F	60	Supraglottic CA	Total laryngectomy and left radical neck dissection
HIn	M	50	CA larynx	Total laryngectomy
NIn	M	69	Transglottic CA	Total laryngectomy and thyroidectomy
PSu	M	51	Transglottic CA and diabetes mellitus	Total laryngectomy
ATh	M	61	CA larynx	Total laryngectomy
LLe	M	77	CA pyriform	Total laryngectomy and right radical neck dissection
NTe	F	55	Supraglottic CA	Total laryngectomy and gastric pull-up with pharyngectomy
PIn	M	66	Transglottic CA	Total laryngectomy
LPe	M	69	Transglottic CA	Total laryngectomy and right modified radical neck dissection
NLi	M	67	Transglottic CA	Total laryngectomy and near total thyroidectomy
MUd	M	48	Transglottic CA	Total laryngectomy and left modified radical neck dissection
TDu	M	66	CA base of mouth	Wide excision and mandibulectomy with bilateral supraomohyoid neck dissection
UAm	M	45	CA larynx	Hemilaryngectomy
FDi	M	73	CA pyriform and diabetes mellitus	Total laryngectomy and left radical neck dissection
SJa	M	60	Transglottic CA	Total laryngectomy
LCh	M	68	Transglottic CA	Total laryngectomy and skin advancement flap
SKo	M	75	CA larynx	Total laryngectomy
LLa	M	65	Transglottic CA	Total laryngectomy

Table 9. Initial characteristics in group II patients

Name	Sex	Age	Diagnosis	Operation
SOr	M	43	CA floor of mouth	Wide excision and segmental mandibulectomy
PVi	M	68	CA larynx	Total laryngectomy and right modified radical neck dissection
BKo	M	50	CA larynx	Total laryngectomy
TSr	M	69	CA larynx	Total laryngectomy and Pectoraris major platysma myocutaneous flap
TTh	M	56	Transglottic CA	Total laryngectomy
LKo	M	59	CA larynx	Total laryngectomy
SJe	M	80	CA larynx	Total laryngectomy
SPr	M	41	CA postcricoid	Total laryngectomy and left radical neck dissection with right modified radical neck dissection
SCh	M	53	Transglottic CA	Total laryngectomy
KHa	M	73	CA hypopharynx	Total laryngectomy and right modified neck dissection
LMa	M	64	Transglottic CA	Total laryngectomy
MJi	M	43	CA larynx	Total laryngectomy
SKu	M	59	CA pyriform	Total laryngectomy and left radical neck dissection
MMo	M	70	CA tongue	Hemiglossectomy and right supraomohyoid neck dissection with left radical neck dissection
SSo	M	68	Transglottic CA	Total laryngectomy and right modified radical neck dissection
SRu	M	67	CA larynx	Total laryngectomy
PPu	M	70	CA floor of mouth cervical, node metastasis	Composite resection and Pectoraris major myocutaneous flap with right modified radical neck dissection and segmental mandibulectomy
VKl	M	61	CA larynx	Total laryngectomy
SRu	M	46	CA pyriform	Total laryngectomy and left modified radical neck dissection
OOn	M	65	CA larynx	Total laryngectomy and bilateral modified radical neck dissection

Table 10. Incidence of bacterial contamination in Isocal RTU present in Tetrapak in group IA at the beginning and end of each feeding

Hanging time	Bacterial growth				Bacterial count	Type of bacteria
	Negative		Positive			
hr	*n*	%	*n*	%	*cfu/mL*	-
0	20	100	0	0	-	
12	15	75	5	25	$5.3x10^2$-$35x10^4$	*Acinetobacter calcoaceticus*
16	17	89.47	2	10.53	$4.2x10$ -$3.8x10^4$	

Table 11. Incidence of bacterial contamination in Isocal RTU drawn through the Luer Lok in group IA at various time of the same feeding

Hanging time	Bacterial growth				Bacterial count	Type of bacteria
	Negative		Positive			
hr	*n*	%	*n*	%	*cfu/mL*	
0	32	88.89	4	11.11	$1.35x10^2$-$35x10^5$	*Acinetobacter calcoaceticus* *Pseudomonas aeroginosa*
4	20	58.82	14	41.18	2.3x10-$3.3x10^5$	*Klebsiella pneumoniae* *Enterobacter* species
8	15	45.45	18	54.54	3.4x10-$1.6x10^6$	*Acinetobacter lwoffii* *Staphylococcus aureus*
12	6	19.35	25	80.65	3.0x10-$5.4x10^7$	*Serratia marcescens*

Table 12. Incidence of bacterial contamination in Isocal RTU in the containers of group IB at the beginning and end of each feeding

Hanging time	Bacterial growth				Bacterial count	Type of bacteria
	Negative		Positive			
hr	*n*	%	*n*	%	*cfu/mL*	
0*	44	100	0	0	-	
0**	49	98	1	2	3.1x10	*Acinetobacter calcoaceticus*
12**	21	84	4	16	$2.2x10-10^7$	
16**	12	70.59	5	29.41	$7.2x10^2-8.5x10^4$	
24**	16	57.14	12	42.86	$6.2x10-1.52x10^6$	

* From Tetrapak , **Form Dobbhoff bag

Table 13. Incidence of bacterial contamination of the formula drawn through the feeding set in group IB at various time for the same feeding

Hanging time	Bacterial growth				Bacterial count	Type of bacteria
	Negative		Positive			
hr	*n*	%	*n*	%	*cfu/mL*	
0	4	50.00	4	50.00	$3x10^3$-$4x10^4$	*Acenetobacter calcoaceticus*
4	8	25.81	23	74.19	1.26-10^2x10^6	*Pseudomonas aeroginosa*
8	5	16.67	25	83.33	$6x10$-$1.3x10^8$	*Serratia marcescens*
12	6	21.43	22	78.57	$3x10^2$-$1.2x10^8$	

Table 14. Incidence of bacterial contamination of RBF present in the containers at the beginning and at the end of each feeding in group II

Hanging time		Bacterial growth				Bacterial count	Type of bacteria
		Negative		Positive			
		n	%	*n*	%	*cfu/mL*	-
8am	Before*	3	9.09	30	90.91	$3.5 \times 10^{-1.28} \times 10^{4}$	*Acinetobacter calcoaceticus*
	After**	3	9.09	30	90.91	$3.7 \times 10^{-9.5} \times 10^{5}$	*Acinetoabacter anitratus*
2pm	Before*	1	2.78	35	97.22	$3.4 \times 10^{-4.4} \times 10^{4}$	*Bacillus* species
	After**	0	0	33	100	$8.5 \times 10^{-10^{6}}$	*Corynbacterium ulcerans*
5pm	Before*	1	2.94	33	97.06	$4.0 \times 10^{-7.7} \times 10^{4}$	*Corynbacterium hoffmanii*
	After**	0	0	33	100	$3.0 \times 10^{-} > 10^{6}$	*Pseudomonas maltophilia*
							Pseudomonas aeroginosa
							Staphylococcus aureus
							Staphylococcus coagulase negative
							Klebsiella pneumonia

* From the glass bottle

** From the plastic bottle after hanging at the patient's bedside for 1-1 1/2 hrs.

Table 15. Means±SEM of daily energy intake and its distribution in groups I and II

Group	Energy	Protein	Fat	Carbohydrate
	kcal/d	< ---------- *g/d* ---------- >		
I	1808.45±5.66	57.87±0.18	75.96 ± 0.24	227.86 ±0.71
II	1901.50±17.34^{A1}	74.83±0.68^{A1}	66.70 ± 0.62^{A1}	233.46 ± 2.13^{A2}
	kcal/kg/d	< ---------- *g/kg/d* ---------- >		
I	35.68±0.97	1.15±0.03	1.50±0.04	4.48±0.13
II	35.27±1.48	1.39±0.06^{A1}	1.24±0.05^{A1}	4.33±0.18
	kcal/d	< ---------- *% of total calories* ---------- >		
I	100	12.67±0.04	37.43±0.12	49.9±0.16
II	100	16.33±0.19	32.74±0.30	50.93±0.46

Significant difference from group I : $A1 p<0.0005$, $A2 p<0.05$

Table 16. Means ± SEM of age, height,body weight and BMI in groups I and II

Group	Day	Age	Height	Body weight		BMI
		yr	*cm*	*kg*	*%std*	*kg/m2*
I	0	62.85±1.41	162.85±1.08	52.22±1.58	89.39±2.43	19.63±0.53
	2			51.95±1.45	88.69±2.24	19.54±0.49
	5-13			51.18±1.43^{a1,b1}	87.61±2.18^{a1,b1}	19.28±0.48^{a1,b1}
II	0	60.25±2.50	165.20±0.96	56.03±3.84	93.62±4.52	20.68±1.00
	2			55.31±3.52	92.37±5.10	20.43±1.13
	5-13			54.76±2.82^{a1,b2}	90.60±4.10^{a1,b2}	20.00±0.91^{a1,b2}

Significant difference from D0 : $^{a1}p<0.0005$

Significant difference from D2 : $^{b1}p<0.0005$, $^{b2}p<0.01$

Table 17. Means±SEM of triceps skinfold thickness, mid upper arm circumference and upper arm muscle circumference in patients groups I and II

Group	Day	TST		MUAC		UAMC	
		mm.	*%std*	*cm*	*%std*	*cm*	*%std*
I	0	9.98±0.66	78.10±4.93	25.74±0.55	88.01±1.88	22.61±0.45	89.80±1.77
	2	9.07±0.58	73.48±4.34	25.36±0.52	86.69±1.78	22.51±0.43	89.48±1.67
	5-13	9.49±0.60	76.78±4.46	25.15±0.50^{a3,b3}	86.41±1.71^{a3,b2}	22.37±0.41^{a3,b2}	88.81±1.59^{a3,b2}
II	0	11.25±1.99	89.91±15.95	26.70±0.67	91.23±2.26	23.21±0.37	92.10±1.50
	2	12.51±2.43	100.11±19.41	27.01±0.84	92.33±2.85	23.08±0.46	91.90±1.78
	5-13	10.94±1.77	87.48±14.19	26.21±0.71^{a1,b3}	89.55±2.39^{a1,b3}	22.77±0.35^{a3}	90.44±1.37^{a3}

Significant difference from **D0** : $^{a1}p<0.001$, $^{a2}p<0.01$, $^{a3}p<0.05$

Significant difference from **D2** : $^{b1}p<0.001$, $^{b2}p<0.01$, $^{b3}p<0.05$

Table 18. Means±SEM of body fat, fat-free mass and total body water in group I (n=30), and II (n=20)

Group	Day	Body fat		Fat free mass		Total body water
		g	*%BW*	*kg*	*L*	*%BW*
I	0	13.80±0.88	24.92±0.84	40.20±1.08	30.33±0.89	56.97±0.80
	2	13.65±0.71	25.51±0.91	39.26±1.10^{a3}	28.95±0.84^{a3}	56.62±0.65
	5-13	13.78±0.77	25.32±0.99	38.39±1.08^{a1}	28.39±0.82^{a1}	56.27±0.76
II	0	14.19±1.49	24.21±1.20	42.43±1.76	32.09±1.36	57.30±0.80
	2	13.98±1.63	24.30±1.46	41.34±2.05	31.30±1.58	57.26±0.97
	5-13	13.67±1.29	24.13±1.06	41.13±1.61^{a3}	31.11±1.24^{a2}	57.36±0.72

Significant difference from D0 : $^{a1}p<0.0005$, $^{a2}p<0.01$, $^{a3}p<0.05$

Table 19. Means±SEM of waist and hip circumferences, and waist/hip circumference ratio in patients group I (n=34), and II (n=18)

Group	Day	Waist circumference	Hip circumference	WHR
		< ---------- *cms* ---------- >		
I	0	76.59±1.64	83.86±0.95	0.91±0.01
	2	76.11±1.64	83.59±0.95	0.91±0.01
	5-13	75.98±2.97	83.72±0.87	0.90±0.01
II	0	78.93±2.60	86.44±1.84	0.91±0.01
	2	78.59±3.58	86.66±2.41	0.90±0.02
	5-13	78.01±2.97	85.48±1.94[a2,b3]	0.91±0.02

Significant difference from Do: a $p<0.005$

Significant difference from D2: b $p<0.05$

Table 20. Prevalences of underweignt, acceptable weight, and obesity by BMI in groups I and II at day 0

Protein-calorie status	Group I		Group II	
	n	%	*n*	%
Underweight	23	58.97	11	55.00
grade III	6	15.38	1	5.00
grade II	9	23.08	5	25.00
grade I	8	20.51	5	25.00
Acceptable weight	12	30.77	7	35.00
Obesity	4	10.26	2	10.00
grade I	-	-	1	5.00
grade II	-	-	1	5.00
Total	39	100	20	100

Table 21. Prevalences of underweight, acceptable weight and obesity in groups I and II by BMI at day 2

Protein-calorie status	Group I		Group II	
	n	%	*n*	%
Underweight	25	65.79	9	56.25
grade III	5	13.16	1	6.25
grade II	11	28.95	5	31.25
grade I	9	23.68	3	18.75
Acceptable weight	10	26.32	5	31.25
Overweight	3	7.89	2	12.50
grade I	3	7.89	1	6.25
grade II	-	-	1	6.25
Total	38	100	16	100

Table 22. Prevalences of underweight, acceptable weight and obesity in groups I and II by BMI at day 5-13

Protein-calorie status	Group I		Group II	
	n	%	*n*	%
Underweight	27	67.50	12	60.00
grade III	6	15.00	2	10.00
grade II	10	25.00	5	25.00
grade I	11	27.50	5	25.00
Acceptable weight	12	30.00	6	30.00
Overweight	1	2.50	2	10.00
grade I	1	2.50	1	5.00
grade II	-	-	1	5.00
Total	40	100	20	100

Table 23. Mean±SEM of serum total protein, albumin, transferrin, and RBP levels in groups I and II

Group	Day	Tatal protein	Albumin	Transferrin	RBP
		<---------- g/L ---------->			mg/L
I	0	76.41+1.14	39.70±0.71	2.04±0.07	41.95±2.56
	2	61.82±1.25^{a1}	28.74±0.79^{a1}	1.66±0.06^{a1}	18.35±1.57^{a1}
	5-13	77.02±1.15^{b1}	33.20±0.91^{a1,b1}	2.08±0.07^{b1}	49.66±3.01^{a3,b1}
II	0	71.38±2.91	38.44±1.94	1.77±0.12^{A}	34.74±3.26
	2	60.83±1.09^{a3}	29.90±1.31^{a1}	1.39±0.11^{a3}	16.87±1.84^{a1}
	5-13	75.46±1.94^{b1}	32.72±1.21^{a3,b3}	1.91±0.10^{b2}	52.50±4.75^{a1,b1}

Significant difference from D0 : $a1p<0.0005$, $a2p<0.001$, $a3p<0.01$

Significant difference from D2 : $b1p<0.0005$, $b2p<0.001$, $b3p<0.01$

Significant difference from group 1 at the corresponding period: $Ap<0.05$

Table 24. Means+SEM of net percentage changes in serum transport protein levels between various study periods in groups I and II

Period	Group	Net percentage changes			
		Total protein	Albumin	Transferrin	RBP
D2 vs D0	I	-18.51±1.88	-27.19±2.00	-18.04±2.33	-56.03±2.79
	II	-10.27±7.61	-15.37±8.52	-13.89±5.78	-44.83±6.16
D5-13 vs D0	I	25.77±2.31	16.58±2.64	27.58±3.42	249.15±38.70
	II	21.97±4.50	9.14±4.71	42.75±11.14	290.79±62.00

Table 25. Means±SEM of serum apo A-I and apo B in groups I and II

Group	Day	Apo A-I	Apo B
		<---------- *mg/dL* ---------->	
I	0	120.96±4.90	123.30±5.86
	2	92.57±3.34^{a1}	90.44±4.70^{a1}
	5-13	111.93±3.47^{b1}	110.93±5.07^{a3,b1}
II	0	101.20±6.38^{A3}	109.29±8.41
	2	74.18±5.04^{a1,A2}	72.90±4.72^{a1,A3}
	5-13	113.62±4.91^{b1}	123.25±6.42^{b1}

Significant difference from D0 : $a1p<0.0005$; $a3p<0.05$

Significant difference from D2 : $b1p<0.0005$

Significant difference from group 1 at the corresponding period: $A2p<0.005$, $A3p<0.05$

Table 26. Means±SEM of net percentage changes in apoprotein A-1 and apo B levels between various periods in groups I and II

Day	Group	Net percent changes	
		Apo A-1	Apo B
		<--------- g/dL --------->	
D2 vs D0	I	-21.48±2.47	-20.78±6.74
	II	-23.56±4.40	-27.96±6.15
D6-13 vs D2	I	24.57±4.38	27.91±5.38
	II	63.32±12.17	82.54±13.89^{A1}

Significant difference from group I: $^{A1}p<0.001$, $^{A2}p<0.01$

Table 27. Prevalence of inadequate visceral protein status in groups I and II

Parameter	Day	Group I		Group II	
		n	%	*n*	%
Total protein <65g/L	0	3	7.5	3	15.0
	2	25	62.5	16	80.0
	5-13	2	5.0	2	10.0
Albumin <35g/L	0	5	12.5	5	25.0
	2	35	87.5	19	95.0
	5-13	26	65.0	15	75.0
Transferrin <2g/L	0	16	40.0	14	70.0
	2	32	80.0	16	84.2
	5-13	19	47.5	15	75.0
RBP <35mg/L	0	12	30.0	12	60.0
	2	37	92.5	19	100.0
	5-13	8	20.0	4	20.0
Apo A-I M <111.24mg/dL	0	18	45.0	13	65.0
F <117.68mg/dL	2	37	92.5	18	90.0
	5-13	19	47.5	7	35.0
Apo B M <86.73mg/dL	0	6	15.0	6	30.0
F <81.94mg/dL	2	22	55.0	16	80.0
	5-13	8	20.0	2	10.0

Table 28. Mean±SEM of serum urea nitrogen and creatinine levels in groups I and II

Group	Day	Urea	Creatinine
		mmol/L	*umol/L*
I	0	4.91±0.33	101.60±6.52
	2	3.58±0.34^{a2}	86.50±6.53^{a3}
	5-13	7.29±0.50^{a1,b1}	102.58±7.50^{b1}
II	0	4.88±0.45	94.30±4.18
	2	3.96±0.23^{a4}	82.45±3.76^{a1}
	5-13	7.01±0.57^{a1,b1}	92.47±5.63^{b5}

Significant difference from D0: $^{a1}p<0.0005$; $^{a2}p<0.001$; $^{a3}p<0.005$; $^{a4}p<0.01$

Significant difference from D2: $^{b1}p<0.0005$; $^{b5}p<0.05$

Table 29. Means±SEM of serum uric acid and fasting blood glucose levels in groups I and II

Group	Day	Uric acid	FBG
		umol/L	*mmol/L*
I	0	345.33±20.51	5.42±0.30
	2	233.40±15.10	7.20±0.31^{a1}
	5-13	257.88±17.12^{a1,b3}	5.25±0.17^{b2}
II	0	321.75±23.89	5.21±0.22
	2	266.90±16.00	7.01±0.37^{a1}
	5-13	360.16±27.14^{b2,A}	5.38±0.17^{b2}

Significant difference from D0: $^{a1}p<0.0005$, $^{a4}p<0.05$

Significant difference from D2: $^{b2}p<0.001$, $^{b3}p<0.005$

Significant difference from group I in corresponding period: $^{A}p<0.005$

Table 30. Means±SEM of urinary creatinine and uric acid excretions in groups I and II

Group	Day	Creatinine	Uric acid
		mmol/d	*mmol/d*
I	-1	15.03±1.15	2.11±0.20
	1	15.82±1.06	2.32±0.25
	2-12	14.23±0.88	1.89±0.10
II	-1	16.80±1.59	2.07±0.26
	1	17.33±1.68	2.03±0.35
	2-12	16.80±1.77	2.29±0.23

Table 31. Means±SEM of creatinine and uric acid clearances in groups I and II

Group	Day	Creatinine clearance		Uric acid clearance	
		mL/min	*mL/min/1.73m²*	*mL/min*	*mL/min/1.73m²*
I	-1	108.92±8.88	188.43±15.37	4 .83±0.51	8.35±0.89
	1	141.57±11.60^{a4}	244.91±20.07^{a4}	8.07±0.83^{a2}	13.96±1.44^{a2}
	2-12	108.45±9.05^{b3}	187.62±15.66^{b3}	6.59±1.09	11.39±1.89
II	-1	127.24±13.21	220.12±22.85	5.11±0.72	8.83±1.25
	1	153.21±15.54	265.05±26.89	4.72±0.66^{A}	8.17±1.14^{A}
	2-12	128.09±15.38^{b5}	221.61±26.61^{b5}	5.11±0.76	8.84±1.31

Significant difference from D0: $^{a1}p<0.0005$, $^{a2}p<0.001$, $^{a4}p<0.01$

Significant difference from D2: $^{b3}p<0.005$, $^{b5}p<0.05$

Significant difference from group I in corresponding time : $^{A}p<0.005$

Table 32. Means±SEM of urinary nitrogen excretions in groups I and II

Day	Group I	Group II
	< ------- *g/d* ------- >	
-1	8.04±0.39	9.09±0.76
1	9.33±0.58[a]	10.62±0.80
2-12	8.38±0.30[b]	10.91±1.00[A]

Significant difference from D0 : [a]p<0.05

Significant difference from D2 : [b]p<0.05

Significant difference from group I in corresponding period : [A]p<0.05

Table 33. Means±SEM of nitrogen balance in groups I and II

Group	Day	Nitrogen		
		Intake	Output	Balance
		< ---------- *g/d* ---------- >		
I	1	0	9.33±0.58	-11.33±0.58
	2-12	9.26±0.03	8.38±0.30	-1.12±0.97[b]
II	1	0	10.62±0.80	-12.62±0.80
	2-12	11.97±0.11	10.91±1.00	-0.94±0.97[b]

Significant difference from D2: [b]p<0.0005

Nitrogen balance = N intake - Urinary N -2

Table 34. Means±SEM of plasma carnitine levels in groups I and II

Group	Day	Free carnitine	Acyl carnitine	Total carnitine
		< ---------- *Umol/L* ---------- >		
I	0	56.62±2.84	65.71±4.85	124.86±6.23
	2	46.64±3.27^{a2}	63.65±4.95	110.94±6.23^{a3}
	5-13	60.92±4.05^{b3}	69.99±7.04	131.53±10.08
II	0	40.44±1.80^{A4}	38.46±3.70^{A1}	78.90±4.21^{A1}
	2	37.75±1.86^{A4}	33.34±3.88^{A1}	71.10±3.97^{a4,A1}
	5-13	39.19±2.74^{A1}	32.83±5.38^{A1}	72.02±6.15^{A1}

Significant difference from D0: $^{a2}p<0.001$; $^{a3}p<0.005$; $^{a4}p<0.05$

Significant difference from D2: $^{b3}p<0.005$

Significant difference from group I in corresponding period: $^{A1}p<0.0005$; $^{A4}p<0.05$

Table 35. Means±SEM of net percentage changes in plasma carnitine levels between various study periods in groups I and II

Period	Group	Net percent changes		
		Free carnitine	Acyl carnitine	Total carnitine
D2 vs D0	I	-16.40±5.01	5.59±7.82	-9.48±3.89
	II	-3.03±6.47	-6.21±11.87	-8.40±4.68
D5-13 vs D2	I	49.46±14.74	49.03±24.14	28.0±49.84
	II	10.70±8.20[A]	11.03±13.42	6.35±7.12

Significant difference from group I: [A]$p<0.05$

Table 36. Means±SEM of urinary carnitine excretion in groups I and II

Group	Day	Free carnitine	Acyl carnitine	Total carnitine
		< ---------- *Umol/day* ---------- >		
I	-1	123.16±12.72	117.82±11.59	242.95±21.93
	1	390.93±41.31^{a1}	301.53±32.76^{a1}	692.46±65.52^{a1}
	2-12	109.30±14.64^{b1}	127.12±13.25^{b1}	236.42±25.64^{b1}
II	-1	103.45±19.81	121.83±12.91	225.28+28.30
	1	423.29±63.99^{a1}	281.44±53.64^{a2}	705.23±93.16^{a1}
	2-12	126.00±25.82^{b1}	123.39±17.56^{b2}	240.44±38.19^{b1}

Significant difference from D0: $^{a1}p<0.0001$, $^{a2}p<0.01$

Significant difference from D2: $^{b1}p<0.0001$, $^{b2}p<0.01$

Table 37. Number and percentage of patients in group I and II by urinary total carnitine excretions

Group	Day	Urinary total carnitine,umol/d					
		<150		150-500		>500	
		n	%	n	%	n	%
I	0	12	30.0	25	62.5	3	7.5
	2	0	0	15	37.5	25	62.5
	5-13	16	40.0	19	47.5	5	12.5
II	0	7	35.0	13	65.0	0	0
	2	1	5.0	4	20.0	15	75.0
	5-13	7	35.0	11	55.0	2	10.0

Table 39. Mean±SEM of apparent carnitine balances in groups I and II during recieving enteral formulas

Group	Carnitine intake			Urinary	Apparent
	Preformed	Biosynthesized	Total	carnitine	carnitine balance
	<---------- *umol/d* ---------->				
I	-	28.45±0.09	28.45±0.09	236.42±25.64	-207.97±25.69
II	43.24±0.39	32.26±0.29	75.50±0.69	240.44±38.19	-164.94±37.99

Table 40. Means±SEM of hematological status in groups I and II

Hematological indices/	I			II		
Group/Day	0	2	5-13	0	2	5-13
Hb (g/dL)	12.99±0.24	11.22±0.24^{a1}	11.65±0.25^{a1,b4}	13.14±0.34	11.32±0.46^{a1}	11.29±0.45^{a1}
Hct (%)	39.61±0.71	34.24±0.85^{a1}	36.17±0.87^{a1,b3}	40.07±1.08	35.35±1.15^{a2}	36.0±41.27^{a4}
RBC (10^{12}/L)	5.26±0.81	4.88±0.87^{a1}	4.98±0.92^{a3,b4}	4.29±0.12	3.78±0.11^{a3}	3.82±0.14^{a4}
MCV fL	87.46±2.01	88.83±2.11	89.26±2.28	94.43±1.76	94.68±1.70	96.36±2.24
MCH pg	31.02±1.83	31.29±1.88	31.20±1.88	31.02±0.72	30.39±0.56	30.19±0.71
MCHC g/L	32.81±0.28	32.55±0.30	32.32±0.35	32.74±0.35	32.16±0.43	31.41±0.44^{a4}
WBC	8.61±0.44	11.76±0.61^{a1}	10.93±0.51^{a2}	9.07±0.37	13.40±0.64^{a1}	10.42±0.82^{b3}
Platelet 10^9/L count	319.22±21.67	260.92±14.69^{a2}	479.46±20.81^{a1,b1}	281.74±22.12	269.74±37.70	516.89±49.78^{a1,b1}
Total Lymphocyte count	2.25±0.23	1.69±0.11	2.18±0.10^{b3}	2.28±0.16	2.35±0.66	1.84±0.14

Significant difference from D0: $^{a1}p<0.0005$, $^{a2}p<0.001$, $^{a3}p<0.005$, $^{a4}p<0.05$

Significant difference from D2 : $^{b1}p<0.0005$, $^{b2}p<0.001$, $^{b3}p<0.005$, $^{b4}p<0.05$

Table 41. Means±SEM of serum bilirubin and liver enzyme levels in groups I and II

Group	Day	Bilirubin		SAST	SALT	GGT	AP
		Total	Direct				
		<---------- *mol/L* ---------->		<---------- *U/L* ---------->			
I	0	5.06±0.84	2.80±0.27	28.48±3.17	21.43±2.08	56.15±12.34	70.20±2.73
	2	21.82±1.24^{a1}	2.99±0.34	29.63±1.52	16.85±1.09^{a6}	28.6±53.55^{a4}	83.23±4.82^{a1}
	5-13	12.37±0.68^{a4,b1}	2.21±0.45	41.45±2.58^{a1,b1}	35.95±3.25^{a1,b1}	68.85±8.13^{a4}	83.23±4.82^{a4,b1}
II	0	16.23±1.02	2.06±0.31	29.35±2.32	22.45±3.23	46.9±58.86	74.50±4.99
	2	18.57±1.54	1.94±0.32^{A}	27.55±1.66	17.30±2.12	28.40±3.35	55.05±3.19^{a1}
	5-13	13.75±1.13^{b6}	1.16±0.22^{a6,b6,A}	48.74±5.35^{a3,b2}	48.95±6.37^{a2,b}	125.89±35.17^{a4,b3}	11.53±18.94^{b4}

Significant difference from D0 : $^{a1}p<0.0005$,$^{a2}p<0.001$,$^{a3}p<0.005$,$^{a4}p<0.01$,$^{a5}p<0.05$

Significant difference from D2 : $^{b1}p<0.0005$,$^{b2}p<0.001$,$^{b4}p<0.01$,$^{b5}p<0.05$

Significant difference from group I in the corresponding period: $^{A}p<0.05$

Table 42. Means±SEM of serum mineral levels and carbondioxide content in groups I and II

	I			II		
Mineral/ Group/Day	0	2	5-13	0	2	5-13
	< ---------- *mmol/L* ---------- >					
Sodium	138.35±0.53	133.35±0.57^{a1}	134.98±0.52^{a1,b4}	138.00±1.13	133.20±0.83^{a1}	133.57±0.75^{a4}
Potassium	4.41±0.08	3.83±0.08^{a1}	5.14±0.12^{a1,b1}	4.22±0.10	3.92±0.07^{a6}	4.61±0.14^{a6,b1}
Chloride	103.35±0.77	99.70±0.66	97.48±0.69^{a1,b2}	104.45±1.12	100.05±0.98^{a3}	99.05±0.70^{a1}
CO2 content	25.89±0.45	23.86±0.46^{a1}	25.51±0.46^{b2}	23.66±0.77	21.85±0.48	20.40±7.16^{a6}
Calcium	2.31±0.03	1.99±0.04^{a1}	2.28±0.04^{b1}	2.31±0.06	2.00±0.05	2.13±0.07^{a6,b6}
Phosphorus	1.12±0.05	0.94±0.08^{a3}	1.42±0.07^{a1,b1}	0.90±0.08	0.87±0.08	1.28±0.09^{a3,b1}
Magnesium	1.60±0.06	1.40±0.04^{a2}	1.77±0.05^{a3,b1}	1.53±0.05	1.35±0.06^{a6}	1.60±0.03^{b2}

Significant difference from D0:$^{a1}p<0.0005$,$^{a2}p<0.001$,$^{a3}p<0.005$,$^{a4}p<0.01$, $^{a5}p<0.05$

Significant difference from D2 : $^{b1}p<0.0005$,$^{b2}p<0.001$,$^{b4}p<0.01$,$^{b5}p<0.05$

CHAPTER IV
DISCUSSION

Protein-energy status

PCM is very common in patients with malignant disease. Its most extreme form associated with cancer produces a constellation of signs and symptoms known as cancer cachexia. It is characterized clinically by anorexia and weight loss, resulting in inanition, weakness, organ dysfunction, and ultimately death. In up to two thirds of cancer patients, death may be attribute to progressive tissue wasting. Although the etiologies of PCM in cancer are multiple, the predominant factor is the imbalance between nutrient and energy intake as well as nutrient and energy requirement. Differing histologic types of cancers cause various degrees of anorexia and endogenously produced peptides, eg, tumor necrosis factor (TNF) having profound effect on intermediate metabolism.[37,38]

Preoperative protein-energy status

Patients in both groups were rather homogenous in terms of gender, underlying diseases, and types of operation out of 40 patients in group I, 37(92.5%) were men, whereas out of 20 patients in group II, 19(95%) were men. Thirty-nine patients (97.5%) in group I and 17 patients (85%) in group II were carcinoma of the larynx; these patients underwent laryngectomy **(Tables 7-9).**

Body composition

Based on 2 compartment model, body composition in each patient was assessed as fat and fat-free mass (FFM) which covered water, protein, glycogen, and mineral (osseous and non-osseous). The assessment of fat and FFM is clinically important because they are the major compartments in which energy stores are distributed and FFM contains all the functional component of the body weight. Fat has an energy density of 9.4 kcal/g, and all but 1 to 2 kg is metabolically available during periods of protracted negative energy balance. Protein and glycogen have respective energy densities of 5.65 and 4.1 kcal/g. Half of the energy contained within FFM can usually be used as fuel during long-term semistarvation. FFM is generally accepted as an index of protein nutriture. Changes in FFM over time are assumed to represent alterations in protein balance.[39]

Vanltallie et al7 have proposed to present body composition data inoterms of body fat mass index (BFMI) and FFM index (FFMI) instead of body fat and FFM as percentages of body weight or as absolute weights. BFMI and FFMI are normalized for stature, being patterned after the BMI. They are calculated as follows:

FFMI = FFM in kg/(ht in m)2

BFMI = BFM in kg/(ht in m)2

Thus BMI is eqeal to BFMI+FFMI. Distribution of BMI, BFMI and FFMI in healthy men (Urbana cohort) by age range has been published.40 BFM and FFM were determined by an electromagnetic scanning instrument. The 5th, 15th, 50th, 75th and 95th percentile cutoff points in 55 men aged 45-54 yrs for BFMI were 3.0, 4.1, 6.4, 9.1, and 10.1 kg/m^2 respectively, whereas the corresponding figures for FFMI were 17.5, 17.8, 19.6, 21.6, and 23.0 kg/m^2, and for BMI were 20.5, 21.9, 26.0, 30.7, and 33.1 kg/m^2.

We had measured th body fat in our patients by near-infrared interactance technique[26], as percent of body weight. The absolute BFM in kg was then calculated from the patient's body weight. FFM was derived from body weight in kg-BFM in kg.

The mean age, height, BFM, and FFM at DO in group I were 62.8 yrs, 1.63 m, 13.80 kg and 40.20 kg, respectively, whereas the corresponding figures in group II were 60.2 yrs, 1.65 m, 14.29 kg, and 42.43 kg **(Tables 16 and 18).** Thus BFMI and FFMI in group I were 5.21 and 15.17 kg/m2 whereas the corresponding figures in group II were 5.20 and 15.55 kg/m2. When these figures were compared with Urbana men aged 45-54 yrs40, BFMI in groups I and II were between the 15th and 50th percentiles of BFMI in Urbana men whereas FFMI in both groups were below the 5th percentile of FFMI in Urbana men. Thus the data indicate inadequate protein-energy status in both groups of patients and the severity of protein-energy disorder was tin the same order. This is supported by the findings that there were no significant differences in their body weight, BMI **(Table 16),** TST, MUAC, UAMC **(Table 17),** waist and hip circumferences, WHR **(Table 19),** and prevalences of underweight **(Table 20)** at DO.

Visceral protein status

In this study, serum total protein, albumin, transferrin, RBP, apo A-I, and Apo B were measured to evaluate visceral protein status in pateints before and during recieving tube feedings.

Serum total protein Serum total protein was one of the first biochemical markers employed in assessing human visceral protein status. Serum total protein reflects primarily circulating albumin and globulin. Although circulating albumin levels are typically normal in marasmus and are decreased in kwashiorkor,[41] globulin levels are variable and are influenced by many factors unrelated to nutrition. Chronic infection can produce a dramatic increase in the globulin fraction, particularly immunoglobulin, which may be large enough to mark in reduction in serum albumin so that serum total protein level is normal or high.[3,42] In our study, the mean serum total protein levels in both groups were above 65 g/L **(Table 23).** The prevalence of total protein <65 g/L in groups I and II at Do were 7.5 and 15%, respectively.

Serum albumin Albumin, from week 20 of gestation and throughout life, is the most abundant protein in human serum, representing 55.65% of the total protein. It is synthesized in the liver at a rate that is dependent on protein intake but subject to feedback regulation by the serum albumin level. The molecule contains no carbohydrate and is not stored to any extent in parenchymal cells. Little abumin is filtered through the renal glomeruli, and most of that is reabsorbed by the proximal tubule cells and degraded by their lysosomal enzymes into fragments that are returned to the circulation.[43]

Albumin has a molecular weight of approximately 66,000. The exchangeable albumin pool is about 4 g/kg in women and 10-20% greater in men. Normally only one-third of the total exchangeable albumin resides in the intravascular space whereas the remaining is distributed through the extravascular space in the skin, muscle, and viscera. Fifteen grams of albumin are synthesized and catabolized daily, and newly produced albumin has a half-life of about 17 days.[44]

The chief bilological functions of serum albumin are to transport and store a wide variety of ligands, to maintain the plasma oncotic pressure, and to serve as a source of endogenous amino acids. hypoalbuminemia is very common in many illnesses resulting from one or more of the following factors: inadequate protein intake, protein malabsorption, impaired biosynthesis, altered distribution that may sequester large amount of albumin in an extravascular compartment, protein loss via urine or feces, and increased protein catabolism.[44]

Serum total protein and albumin levels have been used for the longest time in assessing the human protein-calorie status. Serum total protein appear to be of little value as a sensitive index for estimating protein intake or protein nutritional status. Subjects with inadequate protein-calorie status and having hypoalbuminemia may have elevated serum total protein level resulting from and increase in the alpha-globulin fraction, frequently in association with infection and parasitic infestation. In adults, serum total protein levels of less than 60, 60-64, and 65 g/L and above are considered to be deficient, low, and accepable

protein status, albumin levels are less than 28, 28-34, and 35 g/L and above.[45] In most series, serum albumin level of less than 30 g/L correlate indirectly with morbidity and mortality.[46]

Though the mean serum albumin levels of 39.70 and 38.44 g/L in groups I and II, respectively at, DO were still in the acceptable range, 5(12.5%) out 40 patients in group I and 5 (25.0) out of 20 patients in group II had serum albumin levels of <35 g/L **(Table 27)**. These findings indicate impaired visceral protein status existing in these patients. It should be noted that none of the patients exhibited edema.

Serum transferrin Transferrin is glycoprotein with a molecular weight of approximately 76,000[47] and a mean half-life of 8.8 days.[48] Human apotransferrin is synthesized primarily by the liver. The total amount of apotransferrin in humans is about 240 mg/kg, equally distributed between the plasma and extravascular fluids. Apotransferrin is a true iron carrier which mediated iron exchange between body tissues. It is not lost in delivering iron so its turnover is unrelated to the plasma iron turnover. Binding of ferric ion by apotransferrin occurs in parallel with the binding of an anion, normally bicarbonate, in a pocket formed by arginyl, tyrosyl and histidinyl residues.[49,50] Morton and Tavill[51] have shown that the rate of apotransferrin synthesis is inversely related to iron store; serum transferrin is thus increased in iron deficiency and reduced in iron overload but the mechanism of this iron regulation of synthesis is unknown.

In 1968, Antia et al[52] demonstrated that serum transferrin levels were approximately one fifth of normal in Nigerian children

with kwashiorkor. They also demonstrated a correlation between serum transferrin and the severity and prognosis of the child's disease and noted improvement of serum transferrin levels with nutritional therapy. Studies in hospitalized patients have demonstrated a similar sensitivity of serum transferrin to nutritional status and have documented a prognostic value to this measure in these patients.[53] Because transferrin has a shorter half-life and exists in a smaller body pool than albumin, it is probably more sensitive to acute changes in visceral protein status.[42]

The mean serum transferrin in group II at D0 was less than 2 g/L and significantly lower than that in group I **(Table 23)**. The prevalences of serum transferrin <2g/L in group I and II at D0 were 40 and 70%, respectively **(Table 26)**.

Serum RBP RBP is an alpha1-globulin with a molecular weight of approximately 21,000 and synthesized primarily by the liver.[54] It serves a carrier protein for retinol in plasma and circulated in 1:1 molar ratio with prealbumin. Its short half-life of 10 hrs makes serum RBP level quite sensitive to changes in protein status. Smith et al[55] demonstrated significant reductions in serum RBP, prealbumin, and retinal levels in Egyption children with kwashiorkor; repletion with a high protein diet produced significant increases in these serum protein levels within 2 wks. In general, increases in serum RBP and prealbumin levels precede corresponding changes in serum albumin and total protein levels during nutritional repletion.[56] However, protein status is not the only determinant of serum RBP levels. Venkatswamy et al[57] demonstrated that serum RBP levels in Indian children with

vitamin A deficiency did not return to normal until they had been given with vitamin A and a high protein supplement.

The mean serum RBP level in group II at D0 was less than 35 mg/L and significantly lower than that in group I **(Table 23)**. The prevalences of serum RBP <35 mg/L in groups I and II at Do were 30 and 60%, respectively **(Table 27)**.

Serum apo A-I Apo A is the chief protein constituent of HDL and is subdivided into apo A-I and apo A-II with the molecular weight of 28,300 and 17,000, respectively. When these 2 apoproteins exist together as in HDL, apo A-II enhances the lipid binding properties of apo A-I, possibly by direct protein-protein interaction. Another function of apo A-I is to activate lecithin: cholesterol acyltransferase. Out of 6 apoproteins present in HDL, 66 and 20% were apo A-I and apo A-II, respectively. Both apo A-I and apo A-II are present in chylomicrons, VLDL, and LDL in trace amounts only.[58] The mean($\pm$SEM) serum apo A-I in 66 healthy men and 264 healthy women are 146.20$\pm$4.97 and 144.84$\pm$1.80 mg/dL, respectively. Based on mean$\pm$2SD as the cut-off limits, the normal reference intervals of serum apo A-I levels in 66 healthy men are 111.24-192.32 mg/dL whereas the corresponding values in 453 healthy women are 117.68-178.56 mg/dL. (Soipet and Tanphaichitr : Unpublished data). The mean serum apo A-I levels in both groups were lower than healthy adults **(Table 25)**. The prevalences of low serum apo A-I levels in groups I and II were 45 and 65%, respectively**(Table 27)**.

Serum apo B Apo B is heterogenous and mainly consists of apo B100 and apo B48 with the molecular weight of 512,000 and 241,000, respectively. Apo B100 is found mainly in chylomicrons, VLDL and LDL whereas apo B48 is found only in chylomicrons. Apo B100 is the only apoprotein present in LDL and is a ligand for the LDL receptor. In terms of percentage of apo B present in all apoproteins of various lipoproteins, it constitutes 5-20% in chylomicron, 37% in VLDL, and 97% in LDL whereas it is absent in HDL.[58]

The mean (±SEM) of serum apo B level in 66 healthy men and 453 healthy women are 124.59±4.66 and 111.74±1.40 mg/dL, respectively. Based on mean±2SD as the cut-off limits, the normal reference intervals for men and woman are 86.73-162.45 and 81.94-141.54 mg/dL, respectively. (Soipet and Tanphaichitr: Unpublished data) Both groups of our patients had lower serum apo B levels than healthy adults **(Table 25)**. The prevalences of low serum apo B in groups I and II were 15 and 30%, respectively**(Table 27)**.

Based on the aforementioned findings, it can be concluded that both groups had inadequate visceral protein status.

Postoperative protein-energy status

Body composition

All of the patients in both groups underwent operation **(Tables 7-9)**. Thus they were subjected to surgical stress leading to hypermetabolism59,60 Besides, all of them received inadequate nutritional support on the day of operation (DO) and the first postoperative day (D1). On DO, they received only intravenous glucose providing 200-400 kcal whereas on D1 they also received glucose only providing 1000 kcal with some vitamins and minerals **(Table 6)**. This inadequate protein-energy supply in the presence of stress response to surgery had the significant impact on their protein-energy staus evidence by the decreased in their body composition parameters at D2 **(Tables 16-18)**.

Visceral protein status

The significant decreases in serum total protein, albumin, transferrin, RBP, apo A-I, and apo B levels in both groups at D2 **(Tables 23 and 25)** could be due to several mechanisms including lack of protein intake; blood loss during the operation, Protein synthesis; and an increase in protein catabolism. The decrease in serum albumin levels can be related to a reduction in albumin synthesis caused by direct down regulation of albumin gene expression and translation; a rise in albumin catabolism and an increase in microvascular permeability and extravascular sequestration of albumin. When semistarvation is combined with injury, the usual adaptive movement of albumin into the

intravascular compartment is reversed; vascular permeability is increased, albumin is transfered into the extravascular space, and serum levels are not maintained as they are in the absence of physiologic stress.[39] The significant decreases in their serum urea nitrogen and creatinine at D2 **(Tables 28)** are consistent with the lack of protein intake because both urea nitrogen and creatinine are the catabolic products of amino acid metabolism.[43,59] The significant decreases in their serum ALT and GGT at D2 **(Tables 39)** are most likely due to the lack of protein intake because these 2 enzymes are involved in amino acid metabolism[43,59]

The significant decreases in their Hb, Hct and RBC counts at D2 are most likely due to blood loss during the operation; however, the lack of nutrients required for hemoglobin synthesis should also be considered.

The lack of mineral intakes during D0 and D1 explains the significant decreases in their serum sodium, potassium, chloride, calcium, phosphorus, and magnesium levels at D2 **(Table 40)**.

Efficacy of enteral feedings

During D3-D15, both groups of patients received all of the nutrient supply through tube feeding. **Table 15** shows that the mean daily intakes of energy, protein and carbohydrate in group II were significantly higher than those in group I whereas opposite result was observed for their fat intake. However, when these daily dietary intakes were expressed on their body weight basis, only their intakes of protein and fat were significantly different.

Though all of the patients recieved Isocal RTU of RBF for a short period of 3-11 days, the beneficial effects of tube feedings by these 2 formulas on their protein-energy status were evidenced by the following findings : first, there were no further significant decreases in their body fat and fat-free mass **(Table 18)**, and secondly, there were significantly increases in their serum total protein, albumin, transferrin, RBP **(Tables 23 and 24)**, and apo A-I and apo B **(Table 25 and 26)**, urea nitrogen and creatinine levels at D5-13 **(Table 28)**. The latter findings indicate that adequate supply of protein and energy within a short period can improve the visceral protein status. It has been shown that hypoalbuminemia is a reliable indicator of patients who are at increased risk and who are thus most likely to benefit from intensive nutritional evaluation and treatment.[39] The significant increases in serum AST, ALT, and GGT levels at D5-13 **(Table 39)** also refect the stimulation of these enzymatic activities by the adequate supply of protein to the liver and imply the intact function of liver. It should be noted that the changes in biochemical parameters reflecting liver and renal functions in both groups throughout the study were within normal limits.[43,60]

Nitrogen balance

Nitrogen balance, a measure of net change in total body protein mass, is based on the assumption that almost all total body nitrogen is incorporated the over all nitrogen balance. Equation has 5 components:

Nitrogen balance = N intake-(urinary N+fecal N+integumental N+ miscellaneous N losses)

Nitrogen intake is the total amount of N supplied orally or parenterally to an individual. The largest loss of N is in urine. Urinary nitrogen losses for individuals on a general diet are urea nitrogen 80%, and ammonia nitrogen 7.4%, creatinine nitrogen 6.4%, uric acid nitrogen 2-3% and other minor nitrogenous compounds 1-2%. Fecal nitrogen comes from a mixture of desquamated epithelial cells, bacteria, and unabsorbed dietary protein. Humans on protein-free diet, the fecal nitrogen loss is 0.4g/d. The quantity and quality of protein intakes as well as the integrety of intestinal function determine the amount of fecal nitrogen loss. Skin desquamation, sweat, nasal secretions, hair and nail clippings, menstrual flow, blood withdrawal for diagnostic studies, and seminal discharge all account for small integumental and miscellaneous nitrogen losses.[39] For patients, the nitrogen content of body fluid drainage, eg, paracentesis, or nasogastric aspirates, must be considered.[39]

In our study, nitrogen loss was measured only in urine. Thus 2 g of nitrogen was employed to cover fecal and miscellaneous nitrogen losses.[32] The urinary nitrogen excretions at D1 were endogenous loss of protein in both groups during protein-free diet. They also represented nitrogen loss from the increase in protein catabolism due to surgical stress.[7,61] The validity of urinary nitrogen excretion as an index of somatic protein status is also observed in one study evidenced by significantly positive corelation between urinary nitrogen excretions and FFM ($r = 0.2326$, $p<0.005$) as well as urinary nitrogen excretions and UAMC ($r = 0.1780$, $p<0.0005$). The influence of stress on urinary nitrogen excretions was also supported by the significantly positive correlation between urinary nitrogen excretion and body temperature ($r = 0.2549$, $p<0.02$). All of these findings explain the significantly higher urinary nitrogen excretions in group I at D1 than that at D-1. Similar finding was also observed in group II but the difference did not reach statistical significance **(Table 32).**

Table 15 shows that groups II had significantly higher intakes of energy and protein than group I. Group II had higher energy intake of 93 kcal/d and higher protein intake of 15.81 g/d than group I. The significantly higher urinary excretion in group II at D2-12 than group I was at least in part due to the different in their nitrogen intake. Group II had higher nitrogen excretion of 16.96 g/d than group I **(Table 32).** The difference of their urinary nitrogen excretion was equivalent to 15.81 g protein/d.

Based on apparent nitrogen balance (nitrogen intake-nitrogen excretion), both groups exhibited positively apparent nitrogen balance. However, when 2 g nitrogen/d was used for the calculation of nitrogen balance, both groups exhibited slightly negative nitrogen balance **(Table 33)**. The amount of nitrogen deficit was equivalent to 7.0g/d of protein intake for group I and 5.9 g/d for group II. It should be noted that this negative nitrogen balance was mainly due to inadequate protein intake at D2 in both groups: 31.04 g/d for group I and 39.17 g/d for group II.

Creatinine

Creatine is a nitrogenous organic compound participating in cellular energy metabolism and found primarily in muscle. Body creatine is derived from 2 sources, ie, diets and endogenous biosynthesis. The main dietary source of creatine is muscle. For biosynthesized creatine, two steps reactions and 3 amino acids are required. The first step is the formation of guanidinoacetic acid from glycine and arginine; this reaction is catalyzed by the rate limiting enzyme glycine anidino transferase and takes place mainly in the kidney. The second step is the formation of creatine by guanidinoacetic acid accepting the methyl group from S-adenosylmethionine; the reaction is catalyzed by guanidinoacetate methyl transferase, requires ATP, and occurs primarily in the liver. Biosynthesized creatine is released into the circulation and taken up by muscle and other tissues by active process. This active creatine uptake replaces about 2% of the total amount of creatine in muscle each day.[63]

About 98% of the total body creatine pool resides within muscle where it exists in 2 forms, creatine and phosphocreatine. The formation of phosphocreatine requires ATP and is catalyzed by creatine kinase. It is a high energy compound involving in excitation-contraction of muscle. Creatine spontaneously dehydrates to creatinine at a rate of 1.1% per day while phosphocreatine conversion to creatinine is 2.64% per day. Once formed, creatinine diffuses from the cell and ultimately appears in the urine after glomerular filtration, and to a small extent, tubular excretion.[62]

It is now recognized the invalidity of the earlier report on constancy of 24-hr urinary creatinine excretion. Dietary and non-dietary factors can affect urinary creatinine excretion. The dietary factors include amount of protein, creatine, and creatinine intakes. Now-dietary factors which can increase daily urinary creatinine excretion other than the increase in muscle mass are emotional stress with the magnitude of 5-10%, severe infection, high fever or trauma with the magnitude of 20-100%, and second half of the menstrual cycle with the magnitude of 5-10% whereas decreased urinary creatinine excretion is observed in patients with impaired renal function.[67]

The mean+SEM of urinary creatine excretions in 6 healthy men and women on their habitual diets were 11.33±1.21 and 8.46±1.41 mmol/d, respectively (Tanphaichitr, Kanthatasiri, and Leelahagul: Unpublished data). The majority of our patients were men, i.e., 92.5 and 95% of groups I and II **(Table 7-9)**. Thus both groups had higher urinary creatinine excretions than health adults **(Table 30).** However, there were no significant changes in their urinary creatinine excretions during the study. The higher urinary creatinine excretions in group II at D2-12 than group I was most likely due to the difference in their creatine intake, i.e., Isocal RTU was creatine-free whereas RBF contained creatine.

Constancy of endogenous creatinine production and its release into the body fluids at a constant rate, and constancy of serum creatinine levels over the 24 hr of a day, make creatinine a useful endogenous substance whose clearance may be measured as an indicator of glomerular filtration rate. However, a small quantity of creatinine is reabsorbed by the tubules and a small quantity of creatinine appearing in the urine, 7-10%, is due to tubular secretion. The reference values of creatinine clerance in the literature vary considerably. The values cited by Tietz.[43] are 97-137mL/min/1.73m^2 for men and 88-128mL/min/1.73m^2 for women with the age less than 40 yr; the creatinine clearance decreases 6.5mL/min/1.73m^2 per decade. The values cited by Young[60] are 75-125mL/min without age and sex specification and the values are ot adjusted to 1.73m^2.

The mean±SEM of creatinine clearances in 6 healthy men (Tanphaichitr, Kanthatasiri, and Leelahagul: Unpublished data) were 178.33±19.22 mL/min and 308.50±33.26mL/min/1.73m^2, respectively whereas the corresponding figures in 6 healthy women were 120.67±22.00mL/min and 208.75±38.02mL/min/1.73m^2. Thus both groups had lower creatinine clearances expressed as mL/min or mL/min/1.73m2 throughout the study than healthy adults **(Table 31)**. The highest urinary creatinine clearances at D1 in both groups are due to the lowest serum creatinine levels at D2 **(Tables 28 and 30).** Throughout the study serum creatinine levels in both groups were within the normal levels of 50-110umol/L[60].

The overall findings indicate that amino acid-free and creatine free PPN had a significant effect in decreasing seum creatinine level in our patients (Table 28). The mean dicreases in serum creatinine levels at D2 form Do in groups I and II were 14.9 and 12.5%, respectively. On the contrary, therir urinary creatinine excretions at D1 were higher than those at D-1.

These changes should be due to the effects of surgical stress. However, the effects were minor, which was supported by the lack of correlation between body temperatuer and urinary creatinine excretions.

Uric acid

In human, uric acid is the major product of the catabolism of the purine nucleosides, adenosine and guanosine. Purines from catabolism of dietary nucleic acid are converted to uric acid directly. However, the bulk of purines ultimately excreted as uric acid in the urine arises from degradation of endogenous nucleic acids. Figure 1 shows purine metabolism[63]. Reutilization of the major purine bases, adenine, hypoxanthine, and guanine is achieved through salvage pathway in which phosphoribosylation of the free bases causes resynthesis of the respective nucleotide monophosphates. Approximately 75% of uric acid excreted is lost in the urine; most of the remainder is secreted into the gastrointestinal tract, where it is degraded to allantoin and other compounds by bacterial enzymes. Renal handling of uric acid involves glomerular filtration, reabsorption in the proximal convoluted tubule, subsequent secretion of uric acid into the distal portion of the tubule, and further reabsorption in the distal tubule. The net urinary excretion of uric acid is 6-12% of the amount filtered.[43]

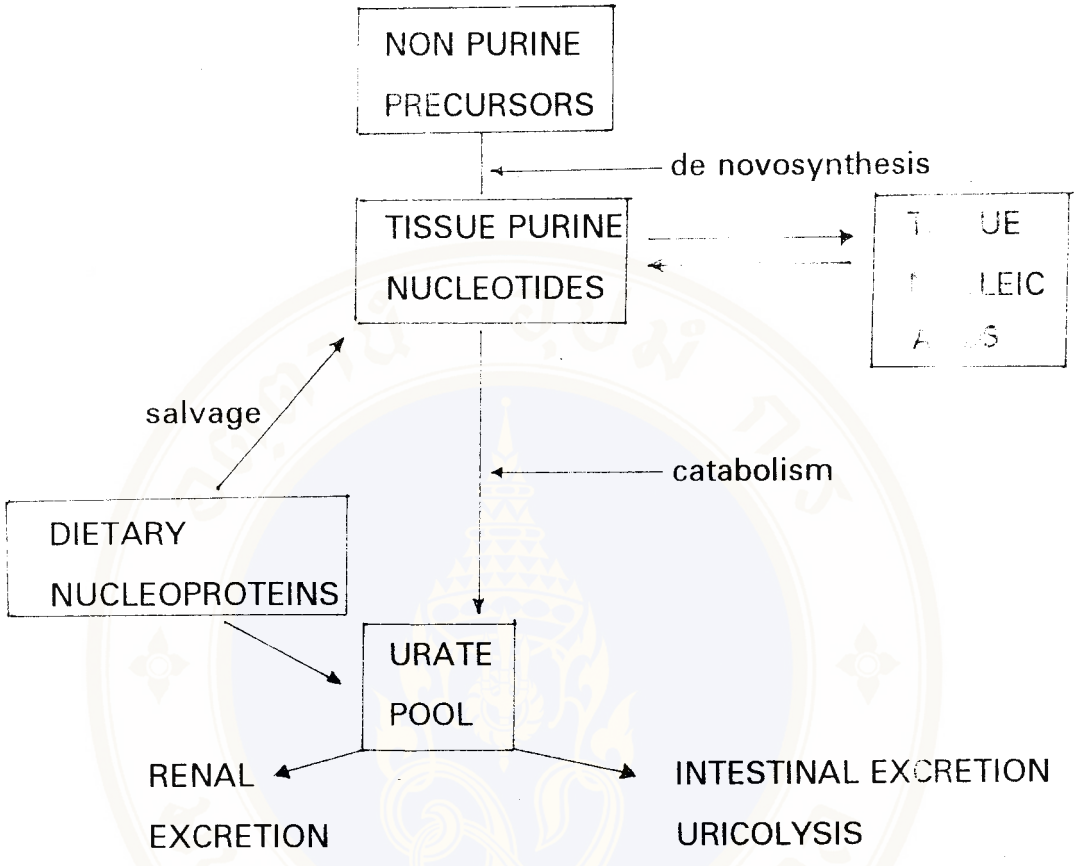

Fig1. Purine metabolism

The serum uric acid value is elevated in an absolute sense when it exceeds the limit of solubility of monosodium urate in serum. At 37°C the saturation value of urate in plasma is about 416.4 umol/L (7.0 mg/dL): a value above this represents supersaturation in a physicochemical sense. The serum uric acid concentration is relatively elevated when it exceeds the upper limit of an arbitary normal range, usually defined as the mean serum uric acid value plus 2 standard deviations in a healthy population matched for age and sex. In most epidemiological studies the upper limit is about 416.4 umol/L (7.0mg/dL) in men and 356.9umol/L (6.0mg/dL) in women. In epidemiological terms a serum uric acid value in excess of 416.4 umol/L (7.0mg/dL) carries an increased risk of gouty arthritis or renal stones.[64] However, according to the Framingham Study, hyperuricemia is defined by serum uric acid levels of ≥368.8umol/L (≥6.2mg/dL) in men or ≥303.3umol/L (≥5.1mg/dL) in women.[65]

Serum and urinary uric acid are influenced by the nucleic acid and protein content in the diet. Purines in the diets on a weight basis will cause a greater increase in uric acid excretion than will dietary proteins. A high purine diet, normally obtained from substantial meat intake, increases the urinary uric acid by approximately 0.5-0.75 mg/mg of ingested purine.[66-69]

When the level of dietary protein is increased the serum uric acid level is lower and the urinary uric acid is higher without and appreciable changes of urinary creatinine excretion.[66-70] A decrease in serum uric acid level of 5.12 umol/L (0.086mg/dL) and an increase urinary uric acid of 41.6 umol/day (7 mg/day) was found for every 10g of protein ingested, based on 2,400 kcal diet.[70] This has been related to an increased renal clearance of uric acid with protein consumption,[71,72] although some studies have shown an increased endogenous production of uric acid at higher levels of dietary protein.[71] The ingestion of amino acids such as alanine, aspartic acid, glutamic acid, or glycine will increase uric acid excretion, possibly by decreasing uric acid reabsorption in the renal tubule.[73,74] Uric acid excretion increased from 1.30 to 2.59 umol/day (218 to 436mg/day) in adults when the dietary protein increased from 0 g/day to 63-75 g/day.[72] However, increased protein ingestion in small increments of 22 to 37 to 75 g/day of egg albumin by healthy male volunteers did not change the average urinary excretion and plasma urate.[72]

Leelahagul and Tanphaichitr (unpublished data) have shown the influence of dietary protein and nucleic acid intakes on urinary urate excretions. The means±SEM of urinary urate excretions in 7 healthy men were 2.62±0.19 mmol/day for their habitual diets (HD) containing 1692±77 kcal and 67±0.4 g of protein, 2.08±0.15 mmol/day for protein frce (PF) containing 1,995±78 kcal and 1.32±0.07 g of protein, 2.86±0.08 mmol/day for vegetarian diet (VD) containing 1,995+78 kcal and 747±2.9 g of protein, 3.81±0.27 mmol/day for regular diet (RD) containing 1,549±55 kcal and 102±1.6 g of protein, and 2.44 mmol/day for

ovovegetarian (OV) diet containg 1,995±78 kcal and 74.7±2.9 g of protein. Their means±SEM of serum urate levels during HB, VD, RD and OV were 302.8±33.9, 265.9±27.4, 303.3±20.8, and 251.6±24.4 umol/L, respectively, whereas the corresponding figures for their urate clearances were 6.88±0.91, 8.9+1.57, 17.1±6.15 and 9.31±1.58 mL/min/1.73m^2. The study of Leelahagul and Tanphaichitr has shown that the highest urinary uric acid excretion was detected during the highest protein intake of the RD period whereas the lowest urinary uric acid excretion was found during the PF period. Besides, there was a significant positive correlation between protein intakes and urinary uric acid excretions ($y=0.325+0.002x$, $r=0.5536$, $df=26$, $t=3.3981$, $p<0.001$). However, VD and OV diets were isocaloric and isonitrogenous urinary uric acid excretion during the VD period was significantly higher than that during the OV period. This was due to the significantly higher purine intake during the VD period than during the OV period, ie, 518±20 vs 414±16 mg/day. The only difference between VD and OV diets was the partial replacement of soybean by egg, a purine-free food, in the OV diet. Their finding that purine intake also influence urinary uric acid excretion is consistent with the reports of Edozien et al[67] and Coe et al.[75] Their study has also shown the positive effect of the amount of protein intake on urate clearance. The urate clearances during the RD, VD, and OV periods were significantly higher than that during the HB period. This explains why serum uric acid levels during the RD and HB periods were not significantly different. However, the higher serum uric acid level during the RD period than during the VD and OV periods was probably due to the higher purine intake derived from the chicken protein during the RD period than during the VD and OV periods. This was supported by the lack of significant difference in uric acid clearance between the RD and VD or OV periods.

Based on the Framingham's criteria, mean serum uric acid levels in both groups were below 368.8 umol/L throughout the study **(Table 29)**. The lowest mean serum uric acid levels in both group at D2 were due to amino acid-free and purine-free PPN. Isocal RTU is purine-free but it contains protein; this explains finding in group I that their mean serum uric acid level at D5-13 was significantly higher than that at D2 but significantly lower than that at D0. RBF contains both purine and protein; this explains the findings in group II that their mean serum uric acid level at D5-13 was significantly higher than that at D2 but significantly lower than that at D0. RBF contains both purine and protein, this explains the findings in group II that their mean serum uric acid level was sgnificantly higher than that at D2 and seum uric acid level in group I at D5-13 **(Table 29).**

Though there were no significant differences in urinary urate excretion between various periods within the same group as well as between the 2 groups at the corresponding periods. The results also suggest the influence of their purine and protein intakes on urinary urate excretions **(Table 30).** The higher urinary urate excretions in group II at D2-12 than that in group I at D2-12 was consistent with the nutritive properties of the 2 enteral formulas, ie, Isocal RTU being purine-free but RBF containing purine. In group I, the highest urinary urate excretion at D1 was consistent with their highest urate and creatinine clearances at D1 **(Table 30 and 31).**

Carnitine status

Carnitine in human body is derived from the intake of preformed dietary carnitine and biosynthesized carnitine stemming from the metabolism of lysine and methionine. Carnitine is syntesized in liver and kidney, stored in skeletal muscle, and mainly excreted in urine. Carnitine has two main functions, ie., transporting long-chain fatty acids into the mitochondrial matrix for B-oxidation to provide cellular energy and modulating the rising in intramitochondrial acylCoA/CoA ratio which relieves the inhibition of many intramitochondrial enzymes involving glucose and amino acid catabolism. Thus the main consequence of carnitine deficiency is impaired energy metabolism. Human carnitine deficiency can be either hereditary or acquired. Hereditary carnitine deficiency can be grouped into 3 clinical entitles: hereditary myopathic carnitine deficiency, hereditary systemic carnitine deficiency, and hereditary organic acidurias. Acquired carnitine deficiency is due to inadequate intake, increased requirement, and increased loss of carnitine. The definite diagnosis of carnitine deficiency is based on the determination of free and acyl carnitine levels in serum, urine and/or tissues. The estimated safe and adequate daily carnitine intake for adults is 150-500 umol/day whereas pharmacologic doses of carnitine are required for the treatment of hereditary carnitine deficiency.[76]

Tanphaichitr and his coworkers[76-83,11,66-71] have shown that the determination of 24-hr urinary carnitine excretion is useful to assess not only protein-calorie and carnitine status but also the presence of protein catabolism in skeletal muscle where most of carnitine resides. Urinary total carnitine excretions in healthy adults, those with protein-calorie malnutrition, and those with catabolism are 150-500, <150 and >500

umol/day, respectively. Based on these cut-off points, 12(30.0%) out of 40 patients in group I at D0 and 7(35.0%) out of 20 patients in group II at D0 had urinary carnitine excretions <150 umol/d whereas 3 patients (75%) in group I and non in group II had urinary carnitine excretions >500 umol/d **(Table 37).** These findings reflect that 19 out of 60 patients with carcinoma of the larynx had inadequate protein-energy and carnitine status whereas 3 patients (5%) were in the state of increased protein catabolism. The influence of surgical stress on urinary carnitine excreton is also illustrated in our study evidenced by the significant increases in urinary free, acyl, and total carnitine excretions at D2 in both groups **(Table 36).** Twenty-five patients (62.5%) in group I and 15 patients (75%) in group II had urinary total carnitine excretion >500 umold/ **(Table 37).** These findings are consistent with the endocrine responses to stress stimuli including infection, fever, tissue injury, and lack of dietary protein and carnitine intake, which lead to the increase in the pituitary production of adrenocorticotrophic hormone followed by the adrenocortical release of cortisol resulting in the increase in urinary carnitine excretions.76-83 Our findings of significantly positive correlations between urinary total carnitine and nitrogen excretions ($r=0.2549$, $p<0.02$) as well as between urinary total carnitine or acyl carnitine and body temperature ($r=0.3039$, $p<0.005$ and $r = 0.3607$, $p<0.005$, respectively) also support to use urinary total carnitine excretion to assess both protein-calorie status and fluctuation of muscle protein catabolism. The highest urinary total carnitine excretions in both groups at D2 agreed with their highest carnitine clearances at D2 **(Table 36 and 38).**

Isocal RTU is a carnitine-free formula[84] whereas RBF contains 17.78 umol/L of carnitine/L, which is derived by calculation from the published data.[77,85] Thus during recieving enteral formulas, carnitine intake in group I depended on biosynthesized carnitine stemming from lysine and methionine[76] whereas carnitine intake in group II was derived from preformed carnitine and biosynthesized carnitine. The mean lysine intakes in groups I and II during recieving Isocal RTU and FBF were 28.45 and 32.26 mmol/d (4.16 and 4.72 g/d), respectively. Based on the studies of Tanphaichitr et al[86,87] showing that 0.1% of lysine was converted to carnitine in the rat, biosynthesized carnitine in groups I and II during recieving enteral formulas was calculated **(Table 39).** Current recommended daily carnitine intake for healthy Thai adult is 150-500 umol/d.[76,88] It is evident that both groups had inadequate carnitine intake, which, in turn, lead to negative carnitine balance **(Table 39).**

The means±SEM of serum free, acyl, and total carnitine levels in 12 healthy adults consisting of 6 men and 6 women are 46.52±2.97, 63.37±6.26, and 109.89±5.31 umol/L (Tanphaichitr et al: Unpublished data)

Table 34 group II exhibited low plasma free, acyl, and total carnitine levels throughout the study. The significant decreases in plasma free carnitine level in group II at D2 could be due to (a) the loss of free carnitine in urine evidenced by significant increase in their urinary free carnitine excretion at D2 **(Table 36)**, and (b) the shift of free carnitine to acyl carnitine because carnitine was utilized for the oxidation of long-chain fatty acid derived from lipolysis due to inadequate energy supply evidenced by non-significant change in plasma acyl carnitine (most likely long-chain acyl carnitine) and

significant increase in urinary acyl carnitine (most likely short-chain acyl carnitine) at D2 **(Tables 34 and 35).**

Since the major site of synthesis of carnitine and visceral proteins, our findings of significantly positive correlations between serum apo A-I ($r=0.2512$, $p<0.001$), apo B ($r=0.2235$, $p<0.005$), serum transferrin ($r=0.2830$, $p<0.001$) or RBP ($r=0.2707$, $p<0.0001$) and plasma carnitine levels suggest that the increased synthesis in these visceral proteins occurred simutaneously with the increase in biosynthesized carnitine.

Microbiological safety

General principle

Enteral feeding can be safe and effective nutritional support method. Its safety depends on the 3 important factors, ie, the choice of the appropriate formula and infusion method, the delivery of the formulas into the appropriate part of the gastrointestinal tract, and the clinical and metabolic evaluation of the patient prior to and during enteral feeding. However, it is now widely recognized that enteral feeds may become contaminated with microorganisms during preparation and administration. This contamination may lead to the development of infectious complications, including bacteremia, septicemia, pneumonia, diarrhea, and infectious enterocolitis.[89]

Potential sources of microbial contamination of enteral feeds include the feed ingredients, kitchen preparations, hospital environment, and physical and technological factors.[90]

Hospital environment. From flower vases to air and dust,[92] considerable varieties of bacterial species have been isolated and cultured in hospitalized patients who are receiving enteral nutrition. One report highlighted how the same microorganisms present on the hands of nurses or patients can be cultured from the enteral diets used.[93]

Physical and technologic factors. Bastow and coworkers showed that if sterile commercial feeds are carefully emptied into diet containers on the hospital unit they remain sterile, whereas if a diet is blended with additives in the diet kitchen it is likely to become contaminated, and subsequent bacterial multiplication occurs (10^9 organisms/ml after 24 hours' exposure to temperatures of 21 to 24°C). It seems likely, therefore, that exogenous sources and mechanisms are responsible for bacterial contamination. Although bacteria can be trapped in nasogastric tubes during insertion[95] and adhesion of microorganisms to the solid surfaces of hardened feeding tubes can occur, some reports show that endogenous bacteria do not contaminate feeding reservoirs in a retrograde fashion when fine-bore nasogastric feeding tubes are used.[96]

With some types of jejunostomy feeding, however, ascending contamination of the reservoir has been suggested.[97] Bacteria from the normal fecal flora can travel through the broadlumen catheter, especially as some of them (*E.coli, Enterobacter cloacae, Proteus, Serratia)* are mobile. Without the use of an infusion pump or antibacterial filter, bacterial invasion of the formula reservoir thus becomes possible. This possibility has been confirmed in clinical studies using closed-diet systems and occurs in up to 25 per cent of patients receiving enteral diet by the nasoenteral, gastrostomy, and jejunostomy routes.[98]

Feed ingredients. Commercially produced diets are mostly sterile and can be given safely. Homemade feeds, however, may be contaminated from the outset. Pasteurized milk may be contaminated with *B cereus*, raw eggs with *Salmonella sp.*, and distilled water with *E. coli, Pseudomonas sp.*, or *Enterobacter sp.*[91]

Kitchen preparations. Even when prepared in the diet kitchen under aseptic conditions contamination can occur during preparation, by mixing and diluting in contaminated machines (mixers, liquefiers, homogenizers) or on working surfaces, or by contaminated staff.[92] Delay in transport to the patient without refrigeration provides a further opportunity for bacterial growth.

Hospital environment. From flower vases to air and dust,[92] considerable varieties of bacterial species have been isolated and cultured in hospitalized patients who are receiving enteral nutrition. One report highlighted how the same microorganisms present on the hands of nurses or patients can be cultured from the enteral diets used.[93]

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Evaluation of microbial contamination

Enteral formulas

It is evident that Isocal RTU in Tetrapak is sterile **(Table 10 and 12).** However, when it was hung for 12 hrs 5(25%) out of 20 samples were bacterial contaminated **(Table 10).**

When Isocal RTU was emptied into Dobbhoff bag, at o hr only 1(2%) out of 50 samples was bacterial contaminated. However, when Isocal RTU in Dobbhoff bag was hung for 12 hrs, 4(16%) out of 25 samples were bacterial contaminated **(Table 12).**

Table 14 clearly shows that RBF was bacterial contaminated prior to the administration with the incidence of 91-97%. This could be explained by the fact that the preparation of RBF was not under aseptic technique. The results are consistent with other report.[99] Our study has also shown that at the end of 1 to 2-hr feeding in the afternoon and evening, all of RBF were bacterial contaminated.

Feeding set

Our study has also shown that the feeding set is also the source of bacterial contamination. Isocal RTU drawn through Luer Lok and the feeding set was bacterial contaminated and also increased with the time for their use **(Table 11 and 13)**.

It should be noted that all the bacterial identified in our study were commonly found in Ramathibodi hospital.

Though none of our patients developed any seriously infectious complications during recieving Isocal RTU or RBF, the results warrant to prevent bacterial contamination of enteral feeds.

CHAPTER V

SUMMARY

The purpose of this study is to evaluate 2 types of enteral formulas, ie., Isocal RTU (a commercial one) and Ramathibodi blenderized formula (RBF: a dietetic-prepared formula) on their microbiological safety and protein metabolism in patients with carcinoma of the larynx.

Sixty patients were divided into 2 groups, ie, group I consisted of 40 patients receiving Isocal RTU whereas group II consisted of 20 patients receiving RBF. Patients in both groups were rather homogeneous in terms of, age, gender, underlying diseases, and types of operation. Their mean(±SEM) age in groups I and II were 62.85±1.41 and 60.25±2.51 yrs, respectively. Most of them were men with carcinoma of the larynx and underwent laryngectomy. Both groups of patients had inadequate protein-energy status and the severity of protein-energy disorder was in the same order and none of the patients exhibited edema. Out of 60 patients, 58 patients received enteral formula via nasogastric tube.

On the day of operation (D0) and the first postoperative day (D1), both groups recieved only partial parenteral nutrition derived from glucose only. Starting from D2 they were tube-fed for 3-11 days (D5-13 postoperatively). The mean daily energy intake in groups I was 1,808 kcal derived from 58 g protein, 76 g fat and 228 g maltodextrins

whereas the corresponding figures in group II were 1,902 kcal, 75 g protein, 67 g fat, and 234 g sucrose.

The results are summarized below.

1. The effects of surgical stress and inadequate nutrition support during D0-1 were evidenced by the finding that both groups at D2, exhibited the deterioration of their protein-energy status evidence by the decreases in their body composition parameters and serum total protein, albumin, transferrin, RBP, apo A-I, apo B, urea nitrogen, ALT and GGT levels. In addition, there were decreases in Hb, Hct, and RBC counts.

2. Even the patients were tube fed for 3-11 days the beneficial effects of Isocal RTU or RBF on protein-energy status evidenced by the following findings, ie, there were no further significant decreases in their body fat and fat-free mass, there were significantly increases in their serum total protein, albumin, transferrin, RBP, apo A-I, apo B, urea nitrogen and creatinine levels at D5-13; and their nitrogen balances were markedly less negative.

3. The significant increases in urinary total carnitine excretion at D1 were due to surgical stress stimuli. Both groups had inadequate carnitine intakes during recieing enteral formulas. This lead to negative carnitine balance in both groups. The positive correlations between plasma carnitine and serum apo A-I, apo-B, transferrin or RBP suggested that the synthesis of these proteins occured simultaneously with the carnitine biosynthesis.

4. Microbiological safety of enteral formulas and feeding sets were assessed. The first 20 patients on Isocal RTU were fed by continuously closed system, the second 20 patients on Isocal RTU by continuously semi-closed system, whereas the remaining 20 patients on RBF were fed by opened intermittent system. Bacterial contamination was determined by pour plate method with dilution before and at the end of each feeding period.

4.1 Isocal RTU in Tetrapak was sterile. After it was hung for 12 hrs, it was bacterial contaminated.

4.2 When Isocal RTU was emptied into Dobbhoff bag, it was bacterial contaminated. After the Dobbhoff bag was hung for 12 hrs, the incidence of bacterial contamination was increased.

4.3 RBF was bacterial contaminated prior to the administration.
At the end of 1 to 1 1/2 -hr feeding, all of RBF were bacterial contaminated.

4.4 The feeding set was also bacterial contaminated.

4.5 All bacteria identified in this study are commonly found at Ramathibodi Hospital.

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