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High-dose insulin administration is associated with hypoaminoacidemia during cardiac surgery

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ABSTRACT

Although the effects of insulin on glucose homeostasis are well recognized in surgical patients, its effect on perioperative protein metabolism has received little attention. The purpose of this study was to examine the effect of high-dose insulin therapy on the plasma concentrations of amino acids (AAs) in patients undergoing coronary artery bypass grafting surgery. We studied 20 nondiabetic patients scheduled for elective coronary artery bypass grafting surgery. Patients were randomly allocated to receive either standard metabolic care (target glycemia 6.0-10.0 mmol/L, control group, n = 10) or high-dose insulin therapy (insulin group, n = 10). Insulin was administered at 5 $mU \cdot kg^{-1} \cdot min^{-1}$ beginning at skin incision. Simultaneously, 20% dextrose was infused at a variable rate adjusted to maintain glycemia between 4.0 and 6.0 mmol/L. Plasma AAs, glucose, cortisol, and insulin were measured immediately before surgery and at sternal closure. Differences in mean values were assessed by Student *t* test. Plasma concentrations of all AAs decreased in the insulin group, with 15 of 22 AAs, including all branched-chain AAs, being significantly lower at sternal closure when compared with the control group. At the end of surgery, plasma glucose concentration was significantly lower in the insulin group (4.2 ± 0.6 vs 7.3 ± 1.0 mmol/L, $P = .0001$), whereas plasma cortisol levels did not show any difference between groups. High-dose insulin therapy resulted in a significant reduction in plasma AAs, particularly branched-chain AAs, during cardiac surgery.

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1. Introduction

Hyperglycemia and loss of body protein are typical features of the catabolic response to open heart surgery [1]. As even moderate increases in circulating blood glucose levels contribute to morbidity and mortality, many patients undergoing cardiac procedures receive insulin for blood glucose control [2,3]. Although the effects of insulin on perioperative glucose homeostasis are well recognized, its influence on amino acid (AA) metabolism is unknown. Amino acids play a key role as precursors for acute phase protein synthesis and are essential for the body's immune response and wound healing [4-7]. In addition, certain AAs are involved in regulating renal blood flow, a clinically important physiologic parameter after cardiac surgery and cardiopulmonary bypass (CPB) [8,9].

The purpose of this study was to examine the effect of high-dose insulin therapy on circulating concentrations of AAs in patients undergoing coronary artery bypass grafting (CABG) surgery. Insulin was administered as part of a hyperinsulinemic-normoglycemic clamp protocol designed to maintain blood glucose levels between 4.0 and 6.0 mmol/L [10].

2. Methods

2.1. Patients

The study protocol was approved by our hospital's Research Ethics Board. Informed consent was obtained from 20 adult patients scheduled for elective CABG procedures requiring CPB between January and September 2006. Severely malnourished (weight loss >20% in preceding 3 months and body mass index [BMI] <20 kg/m^2) and obese (BMI >35 kg/m^2) patients; those with chronic liver disease (abnormal liver function test results, chronic viral hepatitis, or cirrhosis), diabetes mellitus, significantly impaired left ventricular ejection fraction (<40%), or active cancer; and those on dialysis were excluded.

Using a computer program (Plan Procedure; SAS Institute, Cary, NC), consenting patients were randomly allocated to receive the hyperinsulinemic-normoglycemic clamp (insulin group) or standard metabolic care (control group). The study subjects and the personnel analyzing the data were blinded to the treatment.

2.2. Surgical and anesthetic care

Following an overnight fast of at least 8 hours, patients received standard surgical and anesthetic care as established in our institution. Before CPB, heparin 400 U/kg was administered intravenously to obtain an activated clotting time greater than 500 seconds. The ascending aorta and the right atrium were cannulated, and CPB was initiated. Aortic cross-clamp was applied, and cardioplegia was administered. Once the anastomoses were sutured, the aortic cross-clamp was removed, the patient was separated from CPB, and protamine 1 mg/100 U of heparin was administered. The aortic and venous cannulas were removed, hemostasis was established, and the sternum was then closed.

2.3. Study protocol

2.3.1. Control group

Immediately before the induction of anesthesia, a baseline blood glucose value was obtained. Arterial blood glucose was measured every 30 minutes during surgery. If the blood glucose was greater than or equal to 10.0 mmol/L, an insulin bolus (Humulin R regular insulin; Eli Lilly, Indianapolis, IN) of 2 U followed by an infusion of 2 U/h was given. The insulin infusion was then adjusted according to the following sliding scale:

If blood glucose:	Action:
Less than 4.1 mmol/L	stop insulin infusion and administer 25 mL dextrose 5%
4.1 to 6.0 mmol/L	stop insulin infusion
6.1 to 10.0 mmol/L	maintain current infusion rate (2 U/h or more)
Greater than 10.0 mmol/L	increase infusion by 2 U/h

2.3.2. Insulin group

After obtaining a baseline blood glucose value, 2 U of insulin were given as a bolus followed by an insulin infusion of 5 mU·kg^{-1}·min^{-1} (21 U/h for a 70-kg patient). Additional boluses of insulin were given in increments of 2 U for each 2.0-mmol/L increase in blood glucose higher than 6.0 mmol/L. Ten minutes after initiating the insulin infusion and when the blood glucose was less than or equal to 6.0 mmol/L, a continuous infusion of glucose (dextrose 20%) supplemented with potassium (40 mEq/L) and phosphate (30 mmol/L) was administered at a variable rate to maintain normoglycemia (4.0-6.0 mmol/L). The insulin infusion was continued until sternal closure. Arterial blood glucose was measured every 15 to 20 minutes throughout the procedure (Accu-chek glucose monitor; Roche Diagnostics, Basel, Switzerland).

2.4. Metabolic substrates and hormones

Arterial blood samples were drawn before the induction of general anesthesia and at the end of surgery to determine the plasma concentrations of glucose, AA, insulin, and cortisol.

Plasma glucose was measured by a glucose-oxidase method using a Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA). The concentrations of the following 22 plasma AAs were measured by high-performance liquid chromatography using an automated AA analyzer (Biochrom 30; Biochrom, Cambridge, UK): isoleucine, leucine, valine, lysine, methionine, phenylalanine, threonine, tryptophan, alanine, arginine, asparagine, aspartic acid, citrulline, cysteine, glutamic acid, glutamine, glycine, histidine, ornithine, proline, serine, and tyrosine. The analyzer uses Ultropac 8 cation exchange resin and lithium citrate buffers after preparation of a protein-free supernatant by addition of sulfosalicylic acid. Photometric detection with ninhydrin occurs at wavelengths of 570 and 440 nm.

Plasma cortisol and insulin were measured by sensitive and specific double-antibody radioimmunoassays (Amersham International, Amersham, Bucks, UK).

2.5. Statistics

The data were collected using Microsoft (Redmond, WA) Access data entry.

Results are presented as mean ± SD unless otherwise specified, and statistical significance was set as $P < .05$. All P values presented are 2-tailed. Fisher exact test was used to compare categorical variables. Unpaired Student t test was used to assess differences in continuous variables between the control and treatment groups. Paired Student t test was used to assess differences in continuous variables within the same group. All statistical analyses were performed using SPSS 17.0 for Windows (SPSS, Chicago, IL) and PASS 2008 (NCSS, Kaysville, UT).

Based on experiments performed in healthy human volunteers receiving insulin, we expected a difference of at least 40% in branched-chain amino acid (BCAA) levels between the 2 groups. To detect this difference with a type I error of 5% and a power of at least 80%, 10 patients in each group were required.

3. Results

Baseline characteristics and surgical data were similar in the 2 groups (Table 1). No hypoglycemic event (blood glucose value <3.5 mmol/L) occurred during the study period. Four patients in the control group received small doses of insulin (<10 U) during the operation.

Preoperative plasma glucose (control group 6.4 ± 2.1 mmol/L, insulin group 5.9 ± 1.0 mmol/L), insulin (control group 76 ± 30 nmol/L, insulin group 75 ± 41 nmol/L), and cortisol (control group 351 ± 133 μmol/L, insulin group 407 ± 185 μmol/L) concentrations were similar in the 2 groups. At sternal closure, plasma glucose (control group 7.3 ± 1.0 mmol/L, insulin group 4.3 ± 0.6 mmol/L; $P = .0001$) in the insulin group was lower and insulin levels (control 141 ± 83 pmol/L, clamp 3846 ± 966 pmol/L; $P = .0001$) were elevated compared with patients receiving standard care. Plasma cortisol levels decreased intraoperatively without showing any significant difference between groups (control group 185 ± 132 nmol/L, insulin group 245 ± 115 nmol/L).

Plasma AA concentrations were not different between groups at baseline. In the insulin group, plasma concentrations of essential AA (EAA) decreased to a significantly greater extent than in the control group (Table 2). In particular, the BCAA isoleucine and leucine decreased by more than 70% in the insulin group. Patients receiving standard care also showed a small but significant decrease in the levels of isoleucine, valine, phenylalanine, threonine, and tryptophan.

In the presence of insulin, the plasma concentrations of 7 nonessential AAs (NEAAs) decreased to a level significantly lower than that in the control group (Table 3). In the control group, a small but significant decrease of 9 NEAAs (asparagine, aspartic acid, citrulline, glutamic acid, glycine, histidine, ornithine, serine, and tyrosine) was observed.

Table 1 – Characteristics of patients

	Control group	Insulin group
n	10	10
Sex (M/F)	7/3	8/2
Age (y)	64.9 ± 3.8	59.0 ± 9.2
Weight (kg)	78.4 ± 8.1	89.6 ± 19.5
Height (m)	1.67 ± 0.09	1.74 ± 0.12
BMI (kg/m^2)	28.1 ± 2.1	29.5 ± 5.3
β-Blockers (n)	9	9
LVEF (%)	45.5 ± 15.3	51.0 ± 13.5
Surgical time (min)	217 ± 28	198 ± 20
CPB time (min)	91 ± 28	73 ± 25
X-clamp time (min)	78 ± 26	60 ± 22
Crystalloid infusion (L)	3.1 ± 0.7	3.0 ± 0.6

Values are means ± SD or number of patients. There are no significant differences between the groups. M indicates male; F, female; LVEF, left ventricular ejection fraction; X-clamp, aortic cross-clamp.

4. Discussion

The present study demonstrates that insulin administered as part of a hyperinsulinemic-normoglycemic clamp protocol causes significant hypoaminoacidemia in patients undergoing open heart surgery. The plasma concentrations of 15 of 22 AAs, including BCAAs, substantially decreased in the presence of hyperinsulinemia.

Similar findings were reported from studies performed in nonsurgical volunteers demonstrating a lowering effect of insulin on most plasma AAs [11-14]. Using a euglycemic clamp technique with insulin infusions ranging from 6 to 400 $mU \cdot m^{-2} \cdot min^{-1}$ in healthy young men, Fukagawa et al [11] showed a dose-dependent reduction of plasma AA levels. Furthermore, they reported that BCAAs were most sensitive to insulin, demonstrating a 50% to 90% decrease in the presence of hyperinsulinemia. In our study, high-dose insulin, administered at 5 $mU \cdot kg^{-1} \cdot min^{-1}$ (21 U/h in a 70-kg subject or 120 $mU \cdot m^{-2} \cdot min^{-1}$ in a 1.7-m^2 patient) resulting in supraphysiologic plasma insulin concentrations, led to a 70% reduction in BCAA levels.

Major surgical trauma leads to a catabolic state characterized by the imbalance between protein breakdown and synthesis [1]. This stress response is mediated through the neuroendocrine axis, with catecholamines, cortisol, and cytokines involved in activating various pathways of protein degradation. The goal of this response is restoration of adequate perfusion, oxygenation, and importantly the release of substrates for the essential functions of various organs. The main alteration of protein catabolism appears to be an increase in protein breakdown, with the major contribution from skeletal muscle that contains 60% of the body's total protein [15,16]. Muscle protein synthesis after CABG and major abdominal procedures has been shown to decrease, whereas synthesis rates in the liver and the heart appear to increase [17-19].

Amino acids play vital roles in the regulation of basic cellular metabolism. Although AAs seem to be less important under normal physiologic circumstances, they become essential regulators of cardiac adenosine triphosphate production

Table 2 – Plasma EAA concentrations

AA (μmol/L)	Control group		Insulin group		P for differences between groups at sternal closure
	Baseline	Sternal closure	Baseline	Sternal closure	
Isoleucine	56 ± 11	44 ± 14‡	63 ± 5	14 ± 6*	<.001
Leucine	110 ± 18	99 ± 16	123 ± 12	38 ± 14*	<.001
Valine	207 ± 37	186 ± 25‡	233 ± 15	125 ± 18*	<.001
Lysine	188 ± 20	176 ± 23	198 ± 25	128 ± 25*	<.001
Methionine	13 ± 2	15 ± 5	15 ± 4	6.8 ± 2*	<.001
Phenylalanine	49 ± 6	43 ± 8†	53 ± 4	25 ± 6*	<.001
Threonine	102 ± 9	83 ± 15†	115 ± 28	58 ± 15*	<.001
Tryptophan	39 ± 8	22 ± 6*	46 ± 9	15 ± 8*	.043

Values are means ± SD. There are no significant differences in the 2 groups at baseline.
* $P < .001$ for differences between baseline and sternal closure in each group.
† $P < .01$ for differences between baseline and sternal closure in each group.
‡ $P < .05$ for differences between baseline and sternal closure in each group.

during myocardial ischemia and in the postischemic reperfusion period [20]. This applies in particular to BCAAs and AAs that are associated with the malate-aspartate cycle, that is, glutamate, aspartate, arginine, and ornithine, which decreased by 30% in the present protocol [5,6,21-23]. Some AAs have been shown to modulate renal blood flow in the context of cardiac surgery, with arginine, a major precursor of nitric oxide, having the greatest effect [8,9,24,25].

Cysteine is the only AA that significantly increased at the end of the operation in both study groups. This rise in plasma levels may be due to cysteine's unique metabolic properties or a consequence of the systemic inflammatory response induced by the surgical trauma and CPB. Cysteine diminishes free radical activity and is a precursor to glutathione, a powerful antioxidant [4].

Similar to previous observations reporting a 20% reduction in EAAs and NEAAs after CABG, we observed a small but significant decrease in circulating AA concentrations in the control group [17]. This reduction may have been due to altered protein kinetics, that is, changes in protein synthesis and protein breakdown. Alternatively, it could have been caused by the dilutional effects of fluid administration (crystalloids, colloids) as required during surgery and extracorporeal circulation that amounted to an average of about 3 L in our patients.

Glucose-insulin therapy is a metabolic intervention that has been used in cardiac surgery for the last 2 decades and has been found to improve myocardial performance, decrease inotropic support, and improve overall clinical outcomes [26,27]. Hyperinsulinemic-normoglycemic clamp technique is a reliable way to maintain normoglycemia during cardiac surgery while benefiting from the many positive effects of insulin including anti-inflammatory, antifibrinolytic, and cardioprotective properties [10,28-30].

Table 3 – Plasma NEAA concentrations

AA (μmol/L)	Control group		Insulin group		P for differences between groups at sternal closure
	Baseline	Sternal closure	Baseline	Sternal Closure	
Alanine	287 ± 33	320 ± 63	308 ± 51	267 ± 29‡	.027
Arginine	99 ± 10	102 ± 36	110 ± 12	75 ± 19*	.052
Asparagine	44 ± 7	38 ± 11‡	45 ± 8	27 ± 10*	.025
Aspartic acid	20 ± 7	14 ± 5‡	21 ± 8	11 ± 3‡	.122
Citrulline	34 ± 8	25 ± 6*	31 ± 6	15 ± 2*	<.001
Cysteine	1.7 ± 0.4	3.5 ± 1.8*	1.4 ± 0.6	4.2 ± 3.6*	.570
Glutamic acid	215 ± 58	154 ± 57*	217 ± 50	135 ± 49†	.436
Glutamine	315 ± 67	307 ± 75	307 ± 83	245 ± 77†	.084
Glycine	215 ± 57	177 ± 37†	184 ± 45	128 ± 27*	.003
Histidine	69 ± 13	61 ± 12‡	76 ± 19	53 ± 10*	.199
Ornithine	79 ± 11	61 ± 16†	73 ± 19	44 ± 11*	.015
Proline	139 ± 30	142 ± 32	184 ± 56	114 ± 41*	.104
Serine	100 ± 14	68 ± 8*	89 ± 13	42 ± 9*	<.001
Tyrosine	51 ± 6	44 ± 10†	61 ± 17	30 ± 11*	.008

Values are means ± SD. There are no significant differences in the 2 groups at baseline.
* $P < .001$ for differences between baseline and sternal closure in each group.
† $P < .01$ for differences between baseline and sternal closure in each group.
‡ $P < .05$ for differences between baseline and sternal closure in each group.

The clamp also has a significant effect on protein turnover causing considerable hypoaminoacidemia.

The reduction in plasma AAs observed in our study is mainly a reflection of the inhibitory action of insulin on proteolysis. Kinetic studies performed in healthy subjects show that the primary effect of exogenous insulin on whole-body protein metabolism is the suppression of endogenous proteolysis [12,31]. However, insulin has been shown to stimulate muscle protein synthesis and wound healing in burn patients, particularly if hypoaminoacidemia was avoided by the simultaneous provision of AA [32-34]. In an elegant study using leucine methodology, Chevalier and colleagues [34] showed that hyperinsulinemia indeed caused an increase in whole-body protein synthesis when administered with exogenous AA with a view to maintaining postabsorptive plasma AA levels. To achieve this, exogenous AA had to be infused at rates greater than the rate of protein degradation [34]. We postulate that part of the decrease in measured AA levels in the present study may have been due to increased utilization of available AA for protein synthesis because the metabolic effects of insulin are known to be organ and tissue specific [35]. The possibility that protein synthesis may be enhanced in specific organs such as the liver and the heart under conditions of exogenous hyperinsulinemia, especially in catabolic or high-stress conditions, may in fact be beneficial for that organ's function and/or healing. For any prolonged benefit to arise from this hyperinsulinemic-normoglycemic clamp technique, exogenous AA would have to be infused concomitantly to avoid the hypoaminoacidemia that we observed. Infusing exogenous AA would then provide the necessary substrate for endogenous protein synthesis, which could possibly benefit certain organs such as the heart recovering from CPB.

In summary, we achieved supraphysiological levels of exogenous insulin while maintaining euglycemia during CABG surgery and measured pre- and postoperative plasma AA levels. Our study demonstrated that high-dose insulin therapy administered in the context of a hyperinsulinemic-normoglycemic clamp protocol during cardiac surgery resulted in a significant reduction in plasma AAs, particularly BCAAs. We acknowledge certain limitations to our study. Although all patients were labeled nondiabetic and did not receive antidiabetic therapy, it is possible that some had undiagnosed diabetes mellitus; and this may have had an impact on protein metabolism. Furthermore, our study design does not explore the underlying mechanism causing the observed decrease in AA levels. Future studies are needed to examine protein kinetics in the context of exogenous hyperinsulinemia and whether it can be modified by the administration of exogenous AAs.

REFERENCES

[1] Wolfe RR. Regulation of skeletal muscle protein metabolism in catabolic states. Curr Opin Clin Nutr Metab Care 2005;8:61-5.

[2] Estrada CA, Young JA, Nifong LW, et al. Outcomes and perioperative hyperglycemia in patients with or without diabetes mellitus undergoing coronary artery bypass grafting. Ann Thorac Surg 2003;75:1392-9.

[3] Gandhi GY, Nuttall GA, Abel MD, et al. Intraoperative hyperglycemia and perioperative outcomes in cardiac surgery patients. Mayo Clin Proc 2005;80:862-6.

[4] Wu G. Amino acids: metabolism, functions, and nutrition. Amino Acids 2009;37:1-17.

[5] Rau EE, Shine KI, Gervais A, et al. Enhanced mechanical recovery of anoxic and ischemic myocardium by amino acid perfusion. Am J Physiol 1979;236:H873-9.

[6] Svedjeholm R, Ekroth R, Joachimsson PO, et al. Myocardial uptake of amino acids and other substrates in relation to myocardial oxygen consumption four hours after cardiac operations. J Thorac Cardiovasc Surg 1991;101:688-94.

[7] MacKay D, Miller AL. Nutritional support for wound healing. Altern Med Rev 2003;8:359-77.

[8] Jeppsson A, Ekroth R, Kirno K, et al. Insulin and amino acid infusion after cardiac operations: effects on systemic and renal perfusion. J Thorac Cardiovasc Surg 1997;113:594-602.

[9] Jeppsson A, Ekroth R, Friberg P, et al. Renal effects of amino acid infusion in cardiac surgery. J Cardiothorac Vasc Anesth 2000;14:51-5.

[10] Sato H, Carvalho G, Sato T, et al. Perioperative tight glucose control with hyperinsulinemic-normoglycemic clamp technique in cardiac surgery. Nutrition 2010;26:1122-9.

[11] Fukagawa NK, Minaker KL, Young VR, et al. Insulin dose-dependent reductions in plasma amino acids in man. Am J Physiol 1986;250:E13-7.

[12] Tessari P, Trevisan R, Inchiostro S, et al. Dose-response curves of effects of insulin on leucine kinetics in humans. Am J Physiol 1986;251:E334-42.

[13] Schauder P, Schroder K, Matthaei D, et al. Influence of insulin on blood levels of branched chain keto amino acids in man. Metabolism 1983;32:323-7.

[14] Caballero B, Wurtman RJ. Differential effects of insulin resistance on leucine and glucose kinetics in obesity. Metabolism 1991;40:51-8.

[15] Welle S. Human protein metabolism. New York: Springer; 1999.

[16] Biolo G, Fleming RY, Maggi SP, et al. Inverse regulation of protein turnover and amino acid transport in skeletal muscle of hypercatabolic patients. J Clin Endocrinol Metab 2002;87:3378-84.

[17] Caso G, Vosswinkel JA, Garlick PJ, et al. Altered protein metabolism following coronary artery bypass graft (CABG) surgery. Clin Sci (Lond) 2008;114:339-46.

[18] Essen P, Thorell A, McNurlan MA, et al. Laparoscopic cholecystectomy does not prevent the postoperative protein catabolic response in muscle. Ann Surg 1995;222:36-42.

[19] Essen P, McNurlan MA, Wernerman J, et al. Uncomplicated surgery, but not general anesthesia, decreases muscle protein synthesis. Am J Physiol 1992;262:E253-60.

[20] Doenst T, Bugger H, Schwarzer M, et al. Three good reasons for heart surgeons to understand cardiac metabolism. Eur J Cardiothorac Surg 2008;33:862-71.

[21] Safer B. The metabolic significance of the malate-aspartate cycle in heart. Circ Res 1975;37:527-33.

[22] Engelman RM, Rousou JA, Flack JE, et al. Reduction of infarct size by systemic amino acid supplementation during reperfusion. J Thorac Cardiovasc Surg 1991;101:855-9.

[23] Svedjeholm R, Hakanson E, Vanhanen I. Rationale for metabolic support with amino acids and glucose-insulin-potassium (GIK) in cardiac surgery. Ann Thorac Surg 1995;59 (Suppl 1):S15–22.

[24] Giordano M, Castellino P, McConnell EL, et al. Effect of amino acid infusion on renal hemodynamics in humans: a dose-response study. Am J Physiol 1994;267:F703-8.

[25] Castellino P, Levin R, Shohat J, et al. Effect of specific amino acid groups on renal hemodynamics in humans. Am J Physiol 1990;258:F992-7.

[26] Lazar HL, Chipkin S, Philippides G, et al. Glucose-insulin-potassium solutions improve outcomes in diabetics who have coronary artery operations. Ann Thorac Surg 2000;70:145-50.
[27] Koskenkari JK, Kaukoranta PK, Kiviluma KT, et al. Metabolic and hemoynamic effects of high-dose insulin treatment in aortic valve and coronary surgery. Ann Thorac Surg 2005;80: 511-7.
[28] Albacker T, Carvalho G, Schricker T, et al. High-dose insulin therapy attenuates systemic inflammatory response in coronary artery bypass grafting patients. Ann Thorac Surg 2008;86:20-7.
[29] Steganga ME, van der Crabben SN, Levi M, et al. Hyperglycemia stimulates coagulation, whereas hyperinsulinemia impairs fibrinolysis in healthy humans. Diabetes 2009;55: 1807-12.
[30] Cottin Y, Lhuillier I, Gilson I, et al. Glucose insulin potassium infusion improves systolic function in patients with chronic ischemic cardiomyopathy. Eur J Heart Fail 2002;4:181-4.
[31] Fukagawa NK, Minaker KL, Rowe JW, et al. Insulin-mediated reduction of whole body protein breakdown. Dose-response effects on leucine metabolism in postabsorptive men. J Clin Invest 1985;76:2306-11.
[32] Gore DC, Wolf SE, Sanford AP, et al. Extremity hyperinsulinemia stimulates muscle protein synthesis in severely injured patients. Am J Physiol 2004;286:E529-34.
[33] Adegoke OA, Chevalier S, Morais JA, et al. Fed-state clamp stimulates cellular mechanisms of muscle protein anabolism and modulates glucose disposal in normal men. Am J Physiol 2009;296:E105-13.
[34] Chevalier S, Gourgeon R, Kreisman SH, et al. The hyperinsulinemic amino acids clamp increases whole-body protein synthesis in young subjects. Metabolism 2004;53: 388-96.
[35] Boirie Y, Short KR, Ahlman B, et al. Tissue-specific regulation of mitochondrial and cytoplasmic protein synthesis rates by insulin. Diabetes 2001;50:2652-8.