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Postabsorptive muscle protein metabolism in type 1 diabetic patients after pancreas transplantation

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Abstract Insulin was shown to induce protein anabolism in vivo mainly by inhibiting proteolysis. Heterotopic pancreas transplantation in type 1 diabetes mellitus is characterized by peripheral hyperinsulinemia due to systemic rather than portal insulin delivery. Therefore, we studied the postabsorptive muscle protein metabolism in type 1 diabetic patients with or without pancreas transplantation. The forearm balance technique was performed in 9 type 1 diabetic patients on exogenous insulin treatment, in 4 type 1 diabetic patients following successful pancreas transplantation and in 6 healthy volunteers. Labelled leucine and phenylalanine were infused to quantify whole-body and muscle protein synthesis, respectively. In the postabsorptive state, whole-body protein synthesis (leucine kinetics) was similar in pancreas-transplanted patients and controls. In contrast, muscle protein synthesis tended to be less negative in pancreas-transplanted patients with respect to type 1 diabetic patients and healthy volunteers. The present data suggest that recipients with peripheral insulin delivery and chronic hyperinsulinemia are characterized by a preferential stimulation of protein synthesis in muscle rather than in the splanchnic district. When insulin was infused acutely, while maintaining euglycemia, the whole-body and muscle protein synthesis rates were approximately halved in type 1 diabetic patients with and without pancreas transplantation. We conclude that pancreas transplantation is able to normalize basal and insulin-stimulated protein metabolism. Chronic hyperinsulinemia counteract steroid-induced protein degradation by means of a mild, but persistent stimulation of muscle protein synthesis.

Key words Pancreas transplantation • Muscle protein metabolism

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Introduction

Muscle wasting is a well known feature of poorly controlled type 1 diabetes mellitus. In the insulin deficient state, increased levels of plasma amino acids, increased protein turnover and increased protein oxidation rates have been found [1-3]. Both postabsorptive proteolysis and protein synthesis are increased in poorly controlled type 1 diabetic patients, while these defects are reverted by improving the metabolic control [4-6]. Insulin was shown to be effective in inhibiting proteolysis both in vivo and in vitro [7, 8]. In contrast, no study has demonstrated a direct stimulation of protein synthesis by insulin in vivo, with the exception of that by Biolo et al. who demonstrated a direct stimulatory effect of protein synthesis in the muscle district [8]. An increase of amino acid concentration is usually required to cause a direct stimulation of protein synthesis in the whole body, both skeletal muscle and splanchnic tissues being responsible for the increment of total body protein turnover. Nonetheless it is known that the two body districts contribute differently to whole body protein metabolism particularly in type 1 diabetes mellitus [2]. Recent reports document that insulin spares muscle proteins by decreasing breakdown, without a stimulation of protein synthesis [9-11]. A rise of muscle protein synthetic rate seems more dependent on the concentration of circulating amino acids than on that of insulin [12]. Among possible treatments of type 1 diabetes mellitus, both pancreas transplantation and intensive insulin therapy induce postabsorptive peripheral hyperinsulinemia with a relative hypoinsulinization of the splanchnic district [5, 13]. The hypothesis tested in the pres-

ent study was whether the exposition of the muscle district to long-standing postabsorptive hyperinsulinemia (via intensive insulin treatment or pancreas transplantation) may induce a chronic anabolic condition. Since phenylalanine is not metabolized by muscular tissue, the relative uptake or release is a good index of incorporation into, or release from proteins (protein synthesis and proteolysis).

Materials and methods

Subjects

Nine type-1 diabetic patients on insulin (D), 4 type-1 diabetic patients after combined pancreas and kidney transplantation (Tx) and 6 healthy volunteers (CON) were enrolled in the present study. Demographics of the three study groups are given in Table 1. Type-1 diabetic patients received 3 daily injections of regular insulin plus Lente insulin at bedtime. They were admitted to the Metabolic Unit of San Raffaele Hospital at least 24 hours prior to the study. Lente insulin was withdrawn the night prior to the study. Plasma glucose level was maintained in the 120-190 mg/dl range until 8:00 a.m. of the morning of the study via intravenous boluses of regular insulin (0.5 U each). No pancreas-transplanted patient was on insulin. No recipient had rejection episodes in the 4 months preceding the study. All subjects followed an isocaloric diet containing 250 g carbohydrate and 70-90 g protein per day in the two weeks preceding the study. The purpose and potential risks of the study were explained to all subjects and informed consent was obtained before participation. The study protocol was approved by the Institutional Ethical Committee.

Experimental protocol

All subjects were studied in the postabsorptive state after an overnight fast. Polyethylene catheters (18 G Terumo, Leuven, Belgium) were inserted into a brachial artery and retrogradely into an ipsilateral deep forearm vein for blood sampling. The patency of the catheters was maintained by slow infusion of normal saline. A third catheter was introduced into an antecubital vein in the contralateral arm for infusion of tracers solution, insulin and glucose. The subjects received either a 2.5-h (study 1) or a 5-h (study 2) primed, continuous infusion of L-[ring-2,6-H_3]-phenylalanine (26 μCi, 0.42 μCi/min) and L-[1-^{14}C]leucine (11 μCi, 0.16 μCi/min) in combination with a priming dose of $NaH^{14}CO_3$ (3.5 μCi) to prime the body bicarbonate pool. All tracers were obtained from Amersham, Arlington Heights, USA.

Study 1: postabsorptive muscle protein metabolism. All patients participated in this study protocol. The study lasted 150 minutes. Paired arterial and deep venous blood samples were obtained at 10-min intervals during the last 30 min of the study, by which time steady-state conditions were reached. At each sampling interval, blood flow was measured by plethysmography (Model E-20, D.E. Hokanson, Bellevue, Washington, USA), after exclusion of blood flow to the hand by means of a pediatric sphygmomanometer cuff inflated around the wrist at approximately 200 mmHg [14]. Plasma flow was calculated correcting the blood flow for the hematocrit value. Forearm volume was measured by water displacement when the patient inserted the forearm into a graduated cylinder. Indirect calorimetry was performed utilizing Sensors-Medics instrumentation (Model 2900, Anaheim, CA) for at least 30 minutes in the postabsorptive state. Four expired air samples were collected in the last 30 minutes of the study into a CO_2 trapping solution (hyamine hydroxide/absolute ethanol/0.1% phenolphthalein 3:5:1, titrated to trap 1 mmol CO_2 per 3 ml solution).

Study 2: euglycemic hyperinsulinemic clamp. Five D and 4 Tx patients participated in this study protocol. After a 150-minute tracer equilibration period, an insulin clamp was performed, lasting 150 minutes. A prime-continuous infusion of insulin (Actrapid HM, Novo Nordisk, Copenhagen) diluted in a solution of normal saline added with patient's blood was administered at the rate of 40 mU/m^2 min to achieve and maintain an increment in plasma insulin concentration of about 75 μU/ml. Plasma glucose was maintained at basal level by the infusion of a 20% glucose solution at a rate adjusted according to the

Table 1 Demographics of study groups. Values are means ± SE

	D (n = 9)	Tx (n = 4)	CON (n = 6)
Age (years)	30 ± 5	46 ± 4	21 ± 2
Duration of diabetes (years)	5.3 ± 1.2	23.2 ± 4.4	–
Transplant age (years)	–	1.90 ± 0.53	–
Insulin dose (U/day)	42.5 ± 4.54	0	–
Cyclosporin (mg/kg day)	–	5.0 ± 0.5	–
Prednisone (mg/day)	–	10 ± 2	–
Plasma glucose (mmol/l)	7.8 ± 0.5**	4.2 ± 0.5	4.6 ± 0.6
HbA_{1c} (%)	7.5 ± 0.5	6.1 ± 0.1	–
Plasma-free insulin (μU/ml)	17.5 ± 3.2*	17.3 ± 5.1*	4.6 ± 0.6
Plasma C-peptide (ng/ml)	0.88 ± 0.20***	2.26 ± 0.10	2.0 ± 0.2

D, diabetics patients; *Tx*, diabetic patients after pancreas and kidney transplantation; *CON*, healthy volunteers
* $p < 0.001$ with respect to CON; ** $p < 0.01$ with respect to Tx and CON;
***$p < 0.001$ with respect to Tx and CON

plasma glucose concentration measured every 5 minutes on the basis of a negative feedback principle. Paired arterial and deep venous blood samples were obtained at 10-min intervals during the last 30 min of both the basal and the insulin clamp periods by which time steady-state conditions were reached. Blood and plasma flows were measured and calculated (respectively) as described for study 1 during insulin infusion. Indirect calorimetry was performed for 30 minutes in the basal and additional 30 minutes at the end of the insulin infusion period. Four air samples for the measurement of the $^{14}CO_2$ excretion rate were collected as in study 1.

Analytical determinations

Plasma leucine and phenylalanine concentrations and specific activities were determined using an HPLC system, and the radioactive counts of leucine and phenylalanine were measured via a b-scintillation counter as previously reported [5]. Expired $^{14}CO_2$ specific activity was measured in a b-scintillation counter by measuring the radioactivity in known volumes of previously titrated trapping solution [5]. Plasma glucose concentration was measured at the bedside by a Glucose Analyzer 2, Beckman Instruments, Fullerton, CA. Methods for determining plasma free-insulin and C-peptide concentrations have been previously described [5].

Calculations

All measurements were performed in steady-state conditions for both plasma amino acids levels and specific activities. Forearm kinetics of phenylalanine and leucine were calculated according to reported equations [15, 16]. Briefly, the net forearm balance for a given substrate can be calculated when arterial (A) and venous (V) concentrations of the substrate are known together with the forearm plasma flow (F):

$$\text{Net balance} = [(A)-(V)] \times F \quad (1)$$

The net balance for a given amino acid reflects the contribution of tissue net uptake or disposal (Rd) and net release or appearance (Ra) of the substrate:

$$\text{Net balance} = Rd - Ra \quad (2)$$

Tissue disposal (Rd) for leucine and phenylalanine can be calculated from the measured fractional extraction of tracers (E):

$$Rd = E \times (A) \times F \quad (3)$$

where E is the arteriovenous difference in tracer radioactivity ($SA_A - SA_V$) divided by the arterial tracer radioactivity (SA_A):

$$E = (SA_A - SA_V)/(SA_A). \quad (4)$$

Tissue production or release (Ra) of new natural amino acids can be calculated using the difference of net balance and Rd:

$$Ra = (E \times A \times F) - [(A)-(V) \times F] \quad (5)$$

Kinetics of both phenylalanine and leucine were calculated according to these equations. Whole body kinetics data on [^{14}C]leucine turnover were analyzed using a stochastic model previously described [5, 13].

Statistical analysis

All values are expressed as mean ± SE. Comparison between the basal and the insulin-stimulated states within a group was done with the Student's *t* test for paired data. Comparison between the two groups was performed with the ANOVA test.

Results

The arterial plasma concentrations of phenylalanine and leucine were 53.2 ± 5.1 and 114.4 ± 14.1 μM in D, 46.4 ± 10.4 and 94.7 ± 14.5 μM in Tx and 43.6 ± 1.7 and 113.8 ± 4.3 μM in CON, respectively (Table 2). Plasma glucose concentration was higher in D (7.83 ± 0.46 mM) than in Tx (4.19 ± 0.08 mM, $p < 0.01$) and in CON (4.75 ± 0.09 mM, $p < 0.01$). Plasma free insulin level was significantly higher in D (17.5

Table 2 Arterial plasma amino acid concentrations and whole-body phenylananine and leucine kinetics

	D		Tx		CON
	Basal	Insulin	Basal	Insulin	
Free IRI (μU/ml)	17.5 ± 3.2	80.4 ± 3.8	17.1 ± 4.4	76.9 ± 10.0	4.6 ± 0.6
Plasma phenylalanine (mM)	53.2 ± 5.1	45.7 ± 7.9	46.4 ± 10.4	32.1 ± 4.9	43.6 ± 1.7
Plasma leucine (μM)	114.4 ± 14.1	59.4 ± 8.9	94.7 ± 14.5*	46.4 ± 10.4	113.8 ± 4.3
Phe flux μmol/m² min	20.5 ± 3.2	14.5 ± 4.9	17.7 ± 3.7	12.1 ± 1.9	19.9 ± 2.5
ELF μmol/m² min	41.9 ± 5.4	24.9 ± 4.5	40.5 ± 6.0	28.6 ± 0.9	46.4 ± 5.5
LO μmol/m² min	4.9 ± 0.4‡§	3.5 ± 0.6	2.6 ± 0.2§	2.5 ± 0.2	9.0 ± 2.1
NOLD μmol/m² min	37.0 ± 5.1	21.4 ± 4.2	37.9 ± 5.8	26.1 ± 0.9	37.4 ± 4.8

* $p = 0.05$ with respect to CON; ‡ $p < 0.01$ with respect to Tx
§ $p < 0.01$ vs. CON
IRI, immune reactive insulin; *ELF*, endogenous leucine flux; *NOLD*, non-oxidative leucine disposal; *LO*, leucine oxidation

± 3.2 μU/ml) and in Tx (17.1 ± 4.4) than in healthy subjects (4.6 ± 0.6 μU/ml, $p < 0.001$). Plasma C-peptide was markedly reduced in D (0.88 ± 0.2 ng/ml) with respect to Tx (2.26 ± 0.1 ng/ml) and CON (2.0 ± 0.15 ng/ml, $p < 0.001$). The level of plasma-free insulin obtained during study 2 in D (80.4 ± 3.8 μU/ml) was similar to that of Tx (76.8 ± 10.0 μU/ml). During Study 2 the arterial plasma concentrations of phenylalanine and leucine decreased to 45.7 ± 7.9 and 59.4 ± 8.9 μM in D and to 32.1 ± 4.9 and 46.4 ± 10.4 μM in Tx, respectively. These values are similar to levels previously reported for normal subjects in the same experimental condition [11].

Forearm blood flow was significantly lower in the basal state in D (3.3 ± 0.2 ml/min 100 ml forearm) with respect to Tx (4.5 ± 0.2) and CON (4.7 ± 0.4, $p < 0.01$). During insulin infusion, blood flow showed a trend to increase in all groups, persisting a statistically significant difference between diabetic treated by means of exogenous insulin or pancreas transplantation (3.54 ± 0.2 in D and 4.9 ± 0.18 in Tx, $p = 0.001$).

In the postabsorptive state (Study 1), the endogenous leucine flux (ELF) averaged 41.9 ± 5.4 μmol/m² min in D, 40.4 ± 6.0 in Tx and 46.4 ± 5.5 in CON (p = NS among the 3 groups, Table 2). During Study 2, ELF declined to 24.9 ± 4.5 μmol/m² min in D and to 28.6 ± 0.9 in Tx (p = NS). Leucine oxidation (LO), was significantly lower in D (4.9 ± 0.4 μmol/m² min) and in Tx (2.6 ± 0.2) with respect to CON (9.0 ± 2.1, $p < 0.01$) in Study 1. The inhibition of leucine oxidation was similar in the 2 diabetic groups during Study 2 (to 3.5 ± 0.6 and 2.5 ± 0.2 in D and Tx, respectively). The non-oxidative leucine disposal (NOLD, index of protein synthesis) was similar in D, Tx and CON in the basal state (Table 2). During Study 2 hyperinsulinemia reduced NOLD similarly in D (21.4 ± 4.2 μmol/m² min) and in Tx (26.1 ± 0.9).

In the basal state (Study 1), the rates of whole body phenylalanine flux (Table 2) were similar in D, in Tx and in CON (20.5 ± 3.2; 17.7 ± 3.7 and 19.9 ± 2.5 μmol/m² min). During Study 2, the whole-body phenylalanine flux decreased to 14.5 ± 4.9 in D and to 12.1 ± 1.9 in Tx (p = NS).

The net balance for phenylalanine, in the postabsorptive state (Study 1), was 4.0 ± 12.8 nmol/min·100 ml forearm in D, 5.4 ± 6.5 in Tx and -12.3 ± 2.6 in CON (Table 3). During Study 2, the phenylalanine net balance did not change significantly in any of the 2 groups (1.4 ± 8.4 in D and 3.3 ± 6.6 in Tx, p = NS with respect to the basal). The phenylalanine rate of appearance (Phe Ra) tended to be lower in the basal state in D with respect to Tx and CON (13.6 ± 11.7 vs. 25.2 ± 14.3 and 34.2 ± 5.5 nmol/min·100 ml forearm p = NS). In Study 2, Phe Ra did not change significantly in D (10.6 ± 7.8), while it showed a 40% decrease in Tx (14.8 ± 11.2). The phenylalanine rate of disposal (Phe Ra) was similar in the basal state in D and CON (Study 1: 17.6 ± 3.0 and 21.9 ± 4.8 nmol/min·100 ml forearm, respectively) and did not change significantly in D during Study 2 (12.0 ± 1.3). In contrast, in Tx, Phe Rd was higher in the basal state (30.4 ± 10.6; p = NS with respect to D) decreasing to 18.1 ± 4.8 during Study 2.

In the basal state, the net balance for leucine (Table 3) averaged 15.7 ± 9.4 nmol/min·100 ml forearm in D, 7.1 ± 22.3 in Tx and -12.2 ± 7.3 in CON ($p < 0.05$ with respect to D and Tx). During Study 2, the net leucine balance became neutral in D (-0.9 ± 4.9) and did not change in Tx (7.4 ± 13.4). The leucine rate of appearance (Leu Ra) was lower in the basal state in D (15.9 ± 11.3 nmol/min·100 ml forearm, $p < 0.01$) in comparison to Tx and CON (45.9 ± 30.4 and 70.2 ± 11.6, respectively). During the insulin infusion of Study 2, Leu Ra did not change in D (18.6 ± 5.5 nmol/min·100 ml forearm) while it decreased in Tx (25.5 ± 13.4). The leucine rate of disposal (Leu Rd) was not statistically different between the three groups in the basal state (36.2 ± 7.8, 52.9 ± 16.2, 57.9 ± 11.7 nmol/min·100 ml forearm). During Study 1 it decreased both in D (17.6 ± 3.2 nmol/min·100 ml forearm) and Tx (32.8 ± 7.2).

Discussion

We have previously shown that patients affected by type 1 diabetes mellitus are characterized by an increased leucine turnover when in poor metabolic control [5]. We also

Table 3 Forearm phenylalanine and leucine kinetics (μmol/min·100 ml forearm)

	D		Tx		CON
	Basal	Insulin	Basal	Insulin	
Phe Rd	17.6 ± 3.0	12.0 ± 1.3	30.4 ± 10.6	18.1 ± 4.8	21.9 ± 4.8
Phe Ra	13.6 ± 11.7	10.6 ± 7.8	25.2 ± 14.3	14.8 ± 11.3	34.2 ± 5.5
Phe net balance	4.0 ± 12.8	1.4 ± 8.4	5.4 ± 6.5	3.3 ± 6.6	-12.3 ± 2.6
Leu Rd	36.2 ± 7.8	17.6 ± 3.2	52.9 ± 16.2	32.8 ± 7.2	57.9 ± 11.7
Leu Ra	15.9 ± 11.3**	18.6 ± 5.5	45.9 ± 30.4	25.5 ± 13.4	70.2 ± 11.6
Leu net balance	15.7 ± 9.4*	-0.9 ± 4.9	7.1 ± 22.3	7.4 ± 13.4	-12.2 ± 7.3

* $p < 0.05$; ** $p < 0.01$ with respect to CON
Ra, rate of appearance; *Rd*, rate of disappearance

demonstrated that either intensive insulin treatment [5] or pancreas transplantation [13] are capable of normalizing protein metabolism. It is also known that insulin alone induces a positive protein metabolism mainly by inhibiting proteolysis and amino acid oxidation in vivo [1-7] with a direct effect on protein synthesis shown in a single study [8]. In fact, only Biolo et al. were able to show a stimulatory effect of insulin alone on leg muscle protein synthesis [8] in so far as the increment of blood amino acid concentration is a necessary condition to elicit positive protein synthetic rate in humans [4, 5, 12, 13]. Previous work by us showed that the effect of hyperaminoacidemia on whole-body protein synthesis may be due either to a mass action mechanism [4, 5] or to a hormone-like effect of some amino acids (like leucine) acting at the cytoplasmic site and directly activating the p70 S6 kinase, an enzyme involved in the intracellular post-receptorial transmission of the insulin signal [17]. Nonetheless, when patients bearing a benign insulinoma (as a model of chronic hyperinsulinemia) were studied, a trend toward a stimulation of protein synthesis (without any increase in amino acid concentration) in the postabsorptive state was found [18]. Prompted by these results, we undertook the present study with the double aim of (1) assessing protein homeostasis in type 1 diabetes mellitus treated either with exogenous insulin administration or pancreas transplantation (with special focus at the skeletal muscle site) and (2) measuring muscle protein synthesis in a clinical condition characterized by mild chronic hyperinsulinemia (pancreas transplantation with peripheral insulin delivery).

Whole-body leucine turnover (which in the postabsorptive state equals the endogenous leucine flux and therefore proteolysis) was similar in type 1 diabetic patients (either on intensive insulin treatment or after pancreas transplantation) and in healthy volunteers (Table 2). Our present results confirm previous data by us [5, 13] and others [11] and indicate that aggressive treatment of type 1 diabetic patients can restore protein homeostasis. The novel finding of this work is the higher muscle synthetic rate (measured by means of Phe Rd) in pancreas-transplanted patients (with peripheral insulin secretion) with respect to healthy volunteers (with intraportal insulin secretion). This is an important new finding that suggests the mechanism via which chronic hyperinsulinemia conteracts the protein catabolic action of steroid drugs and of other immunosuppressive compounds [13]. The present data also allow us to speculate that given that the muscle synthetic rate is increased and since whole-body protein synthetic rate (NOLD) is not different from the control group, it is concievable that the splanchnic protein synthetic rate of pancreas recipients is lower with respect to that of healthy volunteers.

When insulin was infused, while maintaining euglycemia in diabetic patients, the whole-body protein turnover (both endogenous leucine flux/proteolysis and non-oxidative leucine flux/protein synthesis) was reduced by 40%. Accordingly a 30%-40% reduction in both Phe Ra and Phe Rd was observed, leaving unaltered the overall net phenylalanine balance across the forearm. These data suggest that (1) pancreas transplantation and intensive insulin treatment have a similar effect on the modulation of muscle insulin-mediated protein metabolism (namely, they have a comparable effect on insulin action on body proteins) and (2) the muscle district and the whole body have similar sensitivities to insulin.

In conclusion, pancreas transplantation is capable of normalizing basal and insulin-stimulated protein metabolism. Chronic hyperinsulinemia counteracts steroid-induced protein degradation causing a mild, continuous stimulation of muscle protein synthesis.

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