Transport of Carbon-11-Methionine Is Enhanced by Insulin

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The anabolic effects of insulin are not restricted to carbohydrate and lipid metabolism but also include protein metabolism. However, the effects of insulin on protein metabolism have been difficult to demonstrate in vivo. Amino acid transport is partly regulated by insulin according to the experimental data. PET provides a way to measure fractional uptake rates of amino acids. The purpose of this study was to measure the effect of insulin on amino acid transport from the plasma to the human parotid glands. **Methods:** We compared the uptake of L-[methyl-^{11}C]methionine ([^{11}C]methionine) into the parotid glands and cerebellum in seven healthy volunteers during the fasting state and euglycemic insulin clamp technique (1 mU/kg per minute). **Results:** The fractional uptake rate of [^{11}C]methionine was increased by 31% for the right parotid gland ($p = 0.003$) and by 29% for the left parotid gland ($p = 0.009$) during insulin clamp, while the increase was 19% for the cerebellum ($p = 0.01$). The concentration of amino acids typical for the hormone-sensitive transport system A was 11% lower during insulin infusion than in the fasting state. **Conclusion:** The uptake of methionine into brain tissue does not seem to be under major control by insulin, while the transport of methionine in the parotid glands is stimulated by insulin. PET provides a sophisticated method to study the transport system of amino acids in vivo.

Key Words: PET; insulin; carbon-11-methionine; salivary glands; brain

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PET, with the administration of tracers such as L-[methyl-^{11}C]methionine ([^{11}C]methionine), has made it possible to measure amino acid transport from the plasma to the tissue in vivo (*1*). There are several studies on the amino acid transport systems in vitro, but only a few results have been reported on humans.

Amino acid transport systems across the cell membranes are complex and partly under hormonal regulation. Ubiquitous system A is a sodium ion-dependent strongly energized pump for the transport of most dipolar amino acids and is characterized by hormonal regulation in vitro (*2,3*). Insulin stimulates the transport of amino acids specific for system A in human muscle tissue (*4*), and it has been suggested that the transport of amino acids plays a major role in regulation of protein turnover (*2,4,5*).

A question was raised whether the possible tissue-specific differences in the hormonal control of methionine transport could be measured by PET. Human salivary glands were chosen as the model for studies on transport of amino acids, because our former studies using PET on head and neck cancer patients showed that there is a high normal uptake of [^{11}C]methionine in the salivary glands, and the cerebellum can be studied at the same time as a reference organ (*6*).

Uptake of amino acids is required for the high rate of protein synthesis in the cells of salivary glands. The source of amino acids to be utilized in protein synthesis is the extracellular rather than the intracellular pool of amino acids (*7*). Influx of amino acids has been studied in the cat submandibular gland, which seems to lack system A (*8*), whereas the rat parotid gland has Na^+-dependent uptake of α-aminoisobutyrate, suggesting the presence of system A transport (*9,10*).

The blood-brain barrier is a limiting factor in the transportation of substrates for brain metabolism. It has been suggested that system A is absent in the luminal side of the brain capillary (*11*), while system A type carrier may be located on the antiluminal side transporting short-chain neutral amino acids from the brain to the blood (*12*).

In the current study, we measured the effects of insulin on methionine transport in the human salivary glands and brain using [^{11}C]methionine, PET and the euglycemic insulin clamp technique.

METHODS

Subjects

Seven healthy volunteers (3 women, 4 men; age range 22–64 yr; weighing 62–89 kg, with a body mass index from 21 to 29 kg/m^2), were studied. The subjects were not taking any medication, and had no family history of diabetes mellitus. Each subject gave informed, written consent before participating in the study. The study was approved by the Ethical Committee of the Turku University Central Hospital.

Study Design

All studies were performed after a 10–12-hr overnight fast. Two catheters were inserted, one in an antecubital vein for injection of [^{11}C]methionine and infusion of glucose and insulin, and one in a heated forearm vein for sampling of venous blood. Catheter patency was maintained by a slow infusion of saline. The first PET study was performed in the fasting state. The second PET study was performed at a minimum of 2 hr after the first [^{11}C]methionine injection on the same day or on a separate day within 2 wk under euglycemic hyperinsulinemia using the insulin clamp technique. Blood samples for the measurements of plasma glucose, plasma amino acids, serum insulin and plasma concentration of [^{11}C]methionine were taken as detailed below.

Insulin Clamp Technique

The second PET study began 40 min after starting the euglycemic insulin clamp, which was performed as described previously (*13,14*). A primed-continuous infusion of insulin was used at the rate of 1 mU (or 7.2 pmol)/kg per min. The plasma glucose level was measured (*15*) in duplicate using a glucose analyzer every 5–10 min, and the rate of the 20% glucose infusion was adjusted

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TABLE 1
Plasma Concentrations of System A Amino Acids

	μmol/liter			
	Fast		Hyperinsulinemia	
Amino Acid	0 min	40 min	40 min	80 min
Asparagine	8 ± 7	7 ± 2	7 ± 1	7 ± 3
Threonine	126 ± 16	114 ± 10†	103 ± 16	87 ± 15†
Glycine	276 ± 59	255 ± 63*	239 ± 41	229 ± 42*
Alanine	339 ± 8	274 ± 23†	300 ± 61	284 ± 45
Methionine	23 ± 5	21 ± 5*	18 ± 5	14 ± 4†
Serine	116 ± 18	109 ± 14	97 ± 14	88 ± 16*
Total amino acids	2941 ± 146	2728 ± 217*	2551 ± 250	243 ± 245†

*$p < 0.05$.
†$p < 0.01$ between the start and end of the study.
Plasma concentrations of six amino acids that are typical substrates for system A amino acid transport, and total amino acids during fast and insulin infusion. Data are presented as mean ± s.d.

accordingly to maintain normoglycemia. Serum insulin was measured by an RIA kit (Pharmacia, Uppsala, Sweden), and plasma amino acid concentrations were measured with an LKB-alpha Plus amino acid analyzer (Pharmacia, Uppsala, Sweden) at 20-min intervals.

Measurement of Regional Uptake of Methionine by Salivary Glands and Cerebral Tissue

Carbon-11-methionine was synthesized as described by Långström et al. (*16*), with slight modifications. The radiochemical purity, determined as previously described (*17*), was 97.4% ± 2.0%, the radiochemical impurities being [^{11}C]-D-methionine (2.0% ± 1.0%) and [^{11}C]methionine sulphoxide (1.6% ± 1.0%). An eight-ring ECAT 931/08-tomograph (Siemens/CTI Corp., Knoxville, TN) was used. The device simultaneously produces 15 contiguous sections, with a slice thickness of 6.7 mm and resolution of 6.5 mm in plane (*18*). Transmission scans for attenuation correction were obtained with a removable ^{68}Ge ring source. In each plane, 15–30 × 10^6 counts were collected. Immediately after the transmission scan, [^{11}C]methionine was injected intravenously over 70 sec. Dynamic scanning of the head and neck region was started simultaneously and continued for 40 min (12 × 15 sec, 4 × 30 sec, 3 × 60 sec, 1 × 120 sec, 6 × 300 sec). Frequent blood samples for the measurement of plasma radioactivity were taken and the low molecular weight fraction of plasma taken at 20, 25, 30, 35 and 40 min after the injection was separated by means of gel filtration with Sephadex PD-10 columns (Pharmacia, Uppsala, Sweden) to separate [^{11}C]methionine from its high molecular weight metabolites (*19*).

All data were corrected for deadtime and decay and reconstructed into a 128 × 128 matrix. Elliptical regions of interests (ROIs) were drawn on two or more adjacent cerebral slices, and ROIs of different shapes were placed on the parotid glands on three or more slices so that the whole gland was included.

The tissue time-activity curve and the venous input time-activity curve were analyzed graphically to quantitate the fractional rate of tracer uptake K_i (*20*). Seven points (6.5–30 min) were used to determine the slope by linear regression. A scanning time of 30–40 min from the injection was omitted from the analysis because of the higher amount of metabolites of [^{11}C]methionine at 40 min from the beginning.

Statistical Analysis

All data are presented as mean ± s.d. Statistical comparisons were performed using two-tailed Student's t-test for paired data.

RESULTS

Glucose and Insulin Concentrations

The plasma glucose concentrations were 5.1 ± 0.5 mmol/liter in the fasting state and 5.0 ± 0.5 mmol/liter during insulin infusion (p = ns). The serum insulin concentration was 7.7 ± 5.5 mU/liter in the basal state, while it was significantly increased during the second study (54.8 ± 12.9 mU/liter).

Amino Acid Concentrations

The mean total plasma amino acid concentration was 2813 ± 163 μmol/liter in the basal state. During insulin infusion, the total plasma amino acid concentration was decreased by 13% and averaged 2439 ± 238 μmol/liter (p = 0.0015). The mean concentration of amino acids typical for system A transport was 837 ± 63 μmol/liter in the fasting state and decreased by 11% during the insulin infusion (746 ± 88 μmol/liter; p = 0.02) (Table 1).

There was a clear tendency of individual amino acids to decrease during the scan period (Table 1). The concentration of total amino acids decreased by 8% from the beginning to the end of the study under fasting conditions (p = 0.01). The decrease was 14% during the insulin clamp study (p = 0.00005). However, the relative changes of the total amino acid concentration or six amino acids typical for system A transport were not significantly different between the basal state and hyperinsulinemia, indicating the same decrease of the level of amino acids during the fasting state and hyperinsulinemia.

The fraction of metabolites of [^{11}C]methionine measured by fast gel filtration method was 2.7% ± 2.6% of the radioactivity concentration in the plasma at 20 min after the injection, and it was 12.0% ± 10.3% at 30 min after the injection. The fraction of metabolites increased gradually and was clearly higher at 40 min after the injection (28.1% ± 12.8%). The fractions of metabolites did not differ from each other during the fasting state and hyperinsulinemia.

Methionine Uptake

The fractional uptake rate of [^{11}C]methionine from plasma to parotid gland calculated from 11 to 30 min after the injection was 0.047 ± 0.02 min^{-1} for the right side and 0.049 ± 0.02 min^{-1} for the left side (p = ns). During the insulin infusion, the uptake rate was increased by 31% and 29%, respectively (K_i = 0.062 ± 0.02 min^{-1} for the right side, K_i = 0.064 ± 0.02 for the left side; p = 0.003 and p = 0.009) (Figs. 1 and 2). The fractional uptake rate for cerebellum was 0.030 ± 0.002 min^{-1}

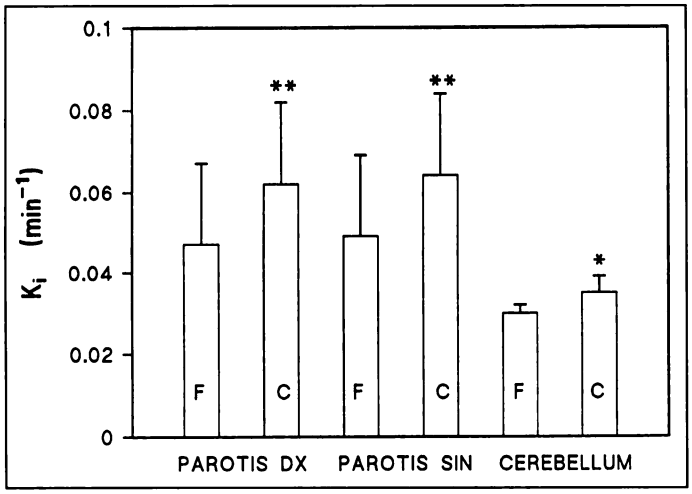

FIGURE 1. Average fractional uptake rates (K_i) of [^{11}C]methionine with s.d. in the right parotid gland (parotis dx), left parotid gland (parotis sin) and cerebellum during the fasting state (F) and insulin clamp (C). *p = 0.01; **p < 0.01.

in the fasting state, while it was 19% higher during hyperinsulinemia (K_i = 0.035 ± 0.004 min^{-1}; p = 0.01).

DISCUSSION

In the current study, we measured fractional uptake rates of [^{11}C]methionine in healthy volunteers in the parotid glands and cerebellum during the fasting state and euglycemic insulin clamp. The uptake rate of [^{11}C]methionine from the plasma to the parotid gland was enhanced by 29%–31% during insulin infusion, while the decrease of the substrate level was only 11%. This result strongly suggests that methionine is transported by an insulin-sensitive transport system from the plasma to the human parotid gland. The stimulation was clearly weaker for brain tissue and, considering the change in the substrate level, the transport of methionine from the plasma to the brain tissue may not be controlled by insulin.

Methionine was first shown to be actively transported by Na^+-dependent and Na^+-independent mechanisms in vitro (*21*). Later on, these mechanisms have been defined to be Na^+-dependent systems A and ASC and Na^+-independent system L (*5*). Systems A and ASC are energy-dependent transport systems to pump amino acids against the concentration gradient from the plasma to the tissues, while system L is forced to operate in reverse (*3*). Hormonally regulated system A has lately been shown to function in human muscle tissue (*4*), while there have been no reports of the transport systems of other human tissues in vivo.

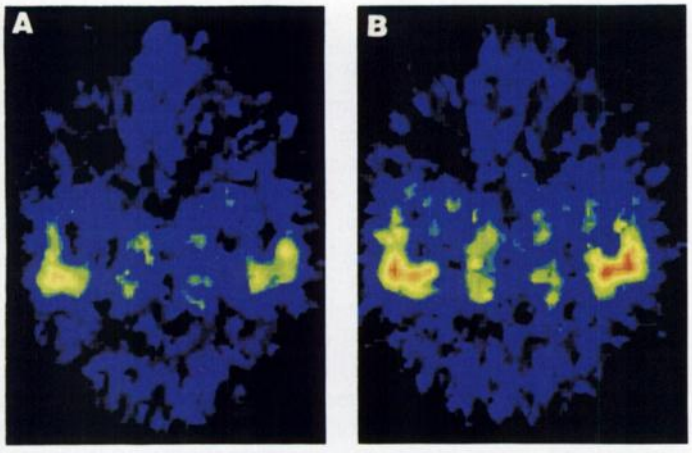

FIGURE 2. (A) Parametric PET image of parotid glands representing fractional uptake rate (K_i) of [^{11}C]methionine. (B) Same image during insulin infusion. The images are scaled in the maximum of the B image.

Insulin is known to increase blood flow in muscle tissue, but the effects of insulin on the blood flow of human salivary glands have not been studied. Autonomic nervous stimulation or per oral lemon juice increase both glandular secretion and blood flow (*22,23*) while the effect of hyperinsulinemia on glandular secretion of salivary glands has not been documented.

Carbon-11-labeled methionine is further metabolized in humans (*24*). Sixty minutes after injection, 40% of the radioactivity is in the protein-bound fraction. However, during the first 30 min, more than 80% of the radioactivity is still [^{11}C]methionine. Thus, the first 30 min of the study after the injection represents [^{11}C]methionine transport (*1*). Because of the nonspecific nature of the transportation and complex metabolism, [^{11}C]methionine cannot be recommended as the tracer of choice for basic physiological studies. An ideal tracer for the studies of amino acid transport systems would not be metabolized during the study. Alfa-methylamino-isobutyric acid labeled with ^{11}C has a high specificity for system A and should be considered for use in further studies to evaluate the activity of system A in vivo (*25,26*).

Insulin infusion induces hypoaminoacidemia (*27,28*). Decrease of the plasma amino acid concentrations also was detected also in the current study, but because of the short evaluation time, the decrease was not advanced considerably. Interestingly, plasma amino acid concentrations also decreased during the fasting study to the same degree as that observed during euglycemic insulin infusion. Special attention should be paid to the scan time and starting point when further studies are planned. On the other hand, doubling of concentrations of amino acids in the plasma by amino acid infusion decreased the fractional uptake rate of 6[^{18}F]fluoro-L-DOPA by 15%–40% (*29*). The moderate change of 11% in the substrate level detected in this study cannot explain the whole change of influx constant values.

CONCLUSION

The uptake of methionine in human salivary glands is regulated by insulin in vivo. Systemic insulin infusion decreased moderately the plasma amino acid concentrations, but the enhancement of the uptake of [^{11}C]methionine in the parotid gland clearly exceeded the decrease of the substrate level. New tracers that evaluate whole amino acid transport systems are warranted. PET enables in vivo studies on amino acid transport systems both in physiological and pathological conditions. The effect of insulin resistance related to, for example, diabetes mellitus and cancer cachexia on the regulation of amino acid transport systems requires more study.

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Fluoride Kinetics of the Axial Skeleton Measured in Vivo with Fluorine-18-Fluoride PET

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The aim of this study was to quantify regional bone blood flow and influx rate with PET and [^{18}F]fluoride in patients with metabolic bone disorders. **Methods:** Dynamic imaging of the spine or pelvis was performed after administration of 300–370 MBq of $^{18}F^-$. Plasma clearance of $^{18}F^-$ was determined in blood sampled from the radial artery. A three-compartment model was used to estimate the regional flow and fluoride influx rate. **Results:** In this preliminary study, fluoride flux (in μmol/min/liter) could be measured regionally. The flux was consistent with the pathophysiology of the studied metabolic disorders and allowed the various disease states to be distinguished. Bone blood flow and influx rate were low in osteoporosis (in the "normal-appearing" bone) and high in Paget's disease. **Conclusion:** With PET and [^{18}F]fluoride, local bone blood flow and fluoride influx rate can be quantified in patients in vivo. Metabolically active zones have an increased influx rate and an accordingly increased flow. In principle, this technique permits classification of bone disorders and has potential for the monitoring of therapy response in metabolic bone disease.

Key Words: ^{18}F; skeletal flow; PET; metabolic bone disease

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The usefulness of $^{18}F^-$ for skeletal imaging was demonstrated decades ago by Blau et al. (*1*), and it subsequently became the standard bone scan in clinical practice (*2*). The uptake of fluoride in bone is rapid and occurs primarily by chemisorption onto hydroxyapatite, followed later by exchange with hydroxyl groups in the hydroxyapatite. After intravenous administration, the radiopharmaceutical accumulates in the perivascular fluid, and the initial $^{18}F^-$ skeletal distribution is regarded as an indicator of blood flow to bone (*3*). In areas of high osteoblastic activity, immature bone is present, which has a large surface area to adsorb the radiopharmaceutical. Charkes et al. (*4,5*) emphasized the difference between skeletal flow and fluoride uptake and mathematically analyzed the contributions of the different compartments. Based on this compartmental model, they elegantly described the implications for routine skeletal scintigraphy in classifying normal and abnormal bone, both in general and in focal disease states (*6*). Using clearance techniques in animals, Wootton et al. (*7–9*) showed that the unidirectional extraction fraction of $^{18}F^-$ in bone was 1 and that the marrow uptake was negligible. Reeve et al. (*10*) found a significant correlation between initial fluoride uptake and Ca^{2+} influx. In addition, two histological indices correlated with the fluoride uptake, i.e., fraction of osteoid taking up double label and corrected apposition rate, revealing the correspondence between fluoride uptake and bone metabolic rate.

PET permits quantification of biochemical processes in vivo and is routinely used in neurological, cardiac and oncological applications (*11,12*). In a previous study from the University of California at Los Angeles (UCLA) (*13*), the application of

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