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Use of positron emission tomography (PET) in the assessment of skeletal muscle glucose metabolism

Anwendung der Positronenemissionstomographie (PET) für die Bestimmung des Glukosemetabolismus des Skelettmuskels

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Summary Non invasive imaging techniques, such as, positronemission tomography (PET), contribute to our present knowledge of glucose metabolism. Besides measurements of net glucose metabolism, PET provides insights into complex processes of intracellular glucose metabolism (i.e., glucose transport and phosphorylation) and is also capable to measure muscular blood flow as a possible determinant of glucose metabolism.

Zusammenfassung Nicht invasive bildgebende Verfahren wie die Positronen-Emissions-Tomographie (PET) erweitern unsere Kenntnisse über den Glukosestoffwechsel. Sie erfassen sowohl die Nettoaufnahme der Glukose in den Muskel als auch die Dynamik des Glukosestoffwechsels (i.e. Transport und Phosphorylierung der Glukose). Darüber hinaus kann die Muskeldurchblutung als mögliche Determinante des Glukosestoffwechsels mit der PET-Technik gemessen werden.

Key words Positron emission tomography (PET) – glucose metabolism – skeletal muscle

Schlüsselwörter Positronenemissionstomographie (PET) – Glukosemetabolismus – Skelettmuskel

Introduction

The in vivo investigation of glucose metabolism has been based on the uses of (i) arteriovenous balance technique (e.g., across the liver, forearm or leg) and (ii) the turnover studies using radioactive and stable isotope tracers for dynamic examination of substrate behavior (Fig. 1). These techniques have been frequently combined with indirect calorimetry or direct tissue assays through the use of biopsies to assess intracellular pathways of glucose metabolism (e.g., glucose oxidation, glycogen synthesis). More recently NMR (=nuclear magnetic resonance) spectroscopy has been used to assess ^{13}C-glucose spectra measuring glycogen metabolism in human liver and skeletal muscle (comp. 1).

Physical imaging techniques like NMR spectroscopy and also positron-emission tomography (PET) directly visualize the behavior of certain molecules in tissues in vivo. Besides measurements of net glucose metabolism, PET adds to our present knowledge since it (i) provides insights into the complex process of intracellular glucose metabolism (i.e., it is possible to calculate glucose transport and glucose phosphorylation (by dynamic PET data)) and thus measures intracellular glucose dynamics and (ii) is capable to assess noninvasively leg blood flow. PET has been only recently applied to the measurement of skeletal muscle glucose metabolism (2-8).

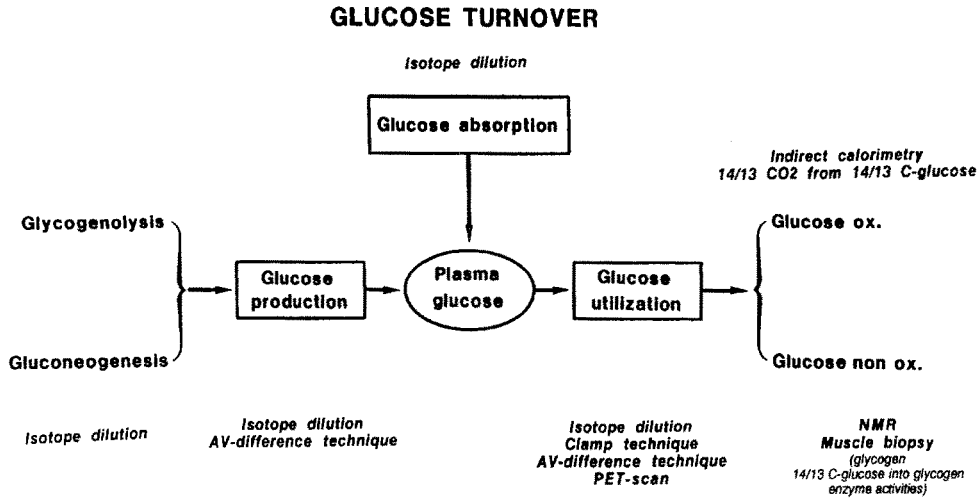

Fig. 1 Schematic presentation of whole body glucose metabolism and different metabolic approaches for it's assessment.

Methodological background

PET was introduced in biomedical research in the mid-1970s. PET is based on the injection of radioactive molecules labeled with positron-emitting nuclides with subsequent tomographic detection of the radioactive nuclide within an organ of interest. Positron emitting isotopes (e.g., ^{15}O, ^{11}C, ^{18}F) can be produced in large quantities in accelerators by specific nuclear reactions. These isotopes are highly labile and disintegrate very rapidly (half life between 2 for ^{15}O, 20 for ^{11}C and 109 min for ^{18}F). Within biological tissues positrons released collide with their antiparticles (i.e., electrons) resulting in the release of two antiparallel, high intensity gamma rays which are only minimally absorbed within tissues. PET takes the advantage of the possibility of measuring the amount of a tracer residing in the muscle by external detection. The positron camera is constructed from rings of gamma ray sensitive scintillation detectors where two x-rays can be detected by coincidence coupling of two scintillation detectors placed at 180^0 to each other. The PET camera produces a tomographic picture with a resolution of the order of 0.5 cm^3. The method considers the tissue as an aggregate of a great number of small regions.

^{18}F-2-deoxy-2-fluoro-D-glucose (^{18}FDG) is conventionally used as a tracer for metabolic imaging and regional assessment of glucose metabolism in brain and heart. In our own PET study on skeletal muscle glucose metabolism 10 mCi ^{18}FDG was injected intravenously during steady state conditions (i.e., after 90 min of an euglycemic clamp protocol, insulin infusion rate: 1.0 mU/kg b.w. x min) (3). ^{18}FDG-activity was measured in arterialized blood and was also traced as a function of time in the mid of thigh by external coincidence counting. PET scan analysis was carried out by a Siemens ECAT 951/31 system. Accurate quantification of regional tissue concentrations of ^{18}FDG has become applicable with the recent development of high spatial and temporal resolution PET-scanners (e.g., of about 5 mm full width at half maximum axial and transaxial) together with advances in computer hardware. In an experiment 31 transaxial slices are recorded simultaneously over time (e.g., over 50 min after injection). The frame rates are 6 times 20 s, 3 times 60 s., 2 times 150 s., 2 times 300 s, and 3 times 600 s. For data analysis the region of interest are placed over the cross-section of the thigh avoiding the vascular and bone area. Counts per sec. are converted to μCi/ml of tissue volume using a calibration bar phantom that approximates the size and volume of human thighs. In addition to tissue activity radioactivity concentrations of arterialized blood samples are recorded by serial sampling at 10 s. intervals for the first 2 min after bolus injection of ^{18}FDG and at increasing time intervals for the rest of the study period. Measurements of blood activity are immediately performed in a calibrated counter. The simultaneous and continuous measurement of disappearance of radioactivity concentrations in blood samples together with the appearance of the tissue radioactivity extends PET from imaging to dynamic studies of metabolism (i.e., PET allows simultaneous determination of glucose transport and phosphorylation and, thus, measures net glucose uptake as well as the rate limiting steps of glucose metabolism in muscle).

A mathematical tracer kinetic model is used to estimate exogenous glucose utilization from PET data. The ^{18}FDG-model is based on the fact that deoxyglucose is transported by the same carriers as glucose and also phosphorylated like glucose. Phosphorylated ^{18}FDG is trapped irreversibly in tissue, whereas ^{18}FDG can go back into the circulation. Data are analyzed by two ways, (i) graphical analysis of blood and tissue time-activity curves for analysis of metabolic rate of glucose (i.e., analysis of

MRGluc according to (9)) and (ii) a three pool compartmental analysis of the fitted data to analyse glucose transport (k1), backflux (k2), and phosphorylation (k3) according to Sokoloff et al. (10). The graphical approach of Patlak (9) does not assume any specific knowledge of the number of compartments that the tracer moves between. It requires that there is free movement of the tracer between any number of compartments and that there is a final compartment in which the tracer is irreversibly trapped. In the Patlak plot the ratio between tissue ^{18}FDG and blood ^{18}FDG concentration is plotted against the integral of the arterialized blood ^{18}FDG concentration from time 0 to time t divided by the blood ^{18}FDG level at time t. After an initial period of ^{18}FDG uptake, the slope of the curve during the time interval is equal to the transfer constant k, which represents the fractional rate of tracer transport and phosphorylation. Then, multiplication of k by the prevailing blood glucose concentration (which should be constant during the study period) gives an index of the metabolic rate of glucose (MRGluc). When compared with the Patlak plot the three compartment model (i.e., (i) a vascular compartment for ^{18}FDG and glucose, (ii) an extravascular tissue compartment that includes interstitial and cellular spaces, and (iii) a cellular compartment for phosphorylated substrates) allows interpretation of the fitted constants k1 transport, k2 for backflux, and k3 for phosphorylation of glucose and ^{18}FDG. The value of the rate constants can be obtained by fitting the differential equation of the ^{18}FDG model to the measured time activity curves. The fit is done using least squares nonlinear regression to fit for the rate constants and the fraction of blood volume. A lumped constant (lc) is used to correct for different metabolic behaviors of the labeled and unlabeled compound (i.e., the tracer and the tracee) and, thus, accounts for differences in the transport and phosphorylation of glucose and ^{18}FDG. In the studies performed so far a lc of 0.67 or 1.00 was used (Comp. 2-8) to account for the differences in the kinetics of transport and phosphorylation of glucose and ^{18}FDG in skeletal muscle. MRGluc was calculated according to glucose concentration / lc x k where k = (k1 x k3)/(k2 + k3). It is evident that the absolute value of MRGluc depends on lc, whereas the kinetic constants are independent of lc. Comparing the MRGluc obtained by Patlak analysis with MRGluc obtained by the compartment model approach shows an excellent agreement with direct curve fitting results (r^2=0.99) (11). This is indirect evidence for the validity of the ^{18}FDG model also in skeletal muscle.

PET-derived muscle glucose metabolism in healthy subjects

Table 1 presents studies published data so far on PET-measurements on skeletal muscle glucose metabolism in healthy subjects as well as in obesity, Type II diabetes mellitus, and in cirrhosis. Although variable, insulin led to a similar stimulation in muscle glucose uptake in all studies, e.g., in healthy controls insulin stimulated glucose uptake was 11.49 ± 2.40 (right thigh) and 12.07 ± 2.56 (left thigh) (data in µmol/100g muscle tissue x min.) (3). Measuring whole body muscle mass by either the urinary creatinine method (35.5 ± 5.1 kg) or anthropometric data (27.0 ± 2.6 kg), it was possible to extrapolate the PET data to whole body glucose uptake (3). There was a significant correlation between clamp- derived whole body glucose uptake (i.e., 45.4 ± 2.9 µmol/kg b.w. x min) and the PET derived data on whole body muscle glucose uptake (r= 0.893 and 0.912, respectively, $p < 0.0001$). The contribution of whole body muscle glucose uptake to whole body glucose disposal was estimated to be 92 ± 25%, which is close to the results obtained by peripheral and splanchnic balance studies (i.e., 96% in a euglycemic clamp study at an insulin infusion rate of 1 mU/kg b.w. x min; comp. 11, 13). PET studies showed values of 3.6 and 2.8 µmol/kg x min (3) and 3.6 and 6.5 µmol/ kg x min for transport and phosphorylation, respectively (3, 8).

To compare the kinetic PET data a recent study using D-mannitol (a nontransportable molecule = tracer 1), 3-O-^{14}C-methyl-D-glucose (transportable, but not metabolizable = tracer 2), and D 3-^{3}H-glucose (transportable and metabolizable = tracer 3) has been used in a euglycemic insulin clamp study combined with the forearm balance

Table 1 Compilation of PET-data characerizing insulin resistance in different clinical situation

	blood flow	glucose metabolism		
		uptake	transport	phosphorylation
obesity	?	↓	↓	=
NIDMM	?	↓	↓	↓
liver cirrhosis	=	↓	↓	=

Table 2 PET-measurements of skeletal muscle glucose metabolism in humans: an overview of published data

author	problem	message
P. Nuutila et al. (2)	„Glucose-Free Fatty Acid-Cycle"	The „Glucose-Free Fatty Acid-Cycle" is operative in man
O. Selberg et al. (3)	Insulin resistance in liver cirrhosis	Cirrhosis impairs muscle glucose uptake and transport without affecting glucose phosphorylation
P. Nuutila et al. (4)	„Glucose-Free Fatty Acid-Cycle"	The „Glucose-Free Fatty Acid-Cycle" is operative in man Insulin resistance
P. Nuutila et al. (5)	in essential hypertension	Insulin resistance is evident in skeletal muscle
P. Nuutila et al. (6)	Effect of gender on insulin sensitivity	Women are more sensitive to insulin than equally fit men
P. Nuutila et al. (7)	Effect of blood flow on insulin sensitivity	Blood flow was not an independent regulator of insulin-stimulated muscle glucose uptake
D.E. Kelley et al. (8)	Effect of obesity/ NIDDM on muscle glucose transport and phosphorylatio	Obesity impairs glucose transport, NIDDM impairs transport and phosphorylation

technique using brachial artery and deep forearm catheterization (14). A number of assumptions and a great body of modelling has been applied in this study and the differences between the washout curves of the three different tracers have been used to calculate glucose transport (i.e., tracer 2-1) and phosphorylation (i.e., tracer 3-2 = hexokinase activity). Using this sophisticated technique in healthy subjects, glucose transport and phosphorylation were calculated to be 59 and 39 μmol/kg x min, respectively (14). The difference may be explained by the data that in contrast to 3-O methyl-D-glucose the k-values of FDG can not be applied directly to glucose since both, transporters and hexokinase, do not handle glucose and deoxyglucose with the same efficiency. At present it has been proposed that dynamic PET provides semiquantitative instead of quantitative data on glucose kinetics (i.e., transport and phosphorylation of glucose) in skeletal muscle.

PET-derived muscle glucose metabolism in disorders of glucose metabolism

Recent PET-data on muscle glucose metabolism assessed in obese, diabetic, and cirrhotic patients are presented in Tables 1 and 2. It is evident that PET may contribute to our present understanding in disorders of glucose metabolism.

Critics and future perspectives of the PET approach

Critics of the PET approach measuring skeletal muscle glucose metabolism deal with the efficiency of handling of glucose and deoxyglucose by hexokinase as well as the unknown influence of an increase in glucose fractional extraction rate on the calculation of the rate constants which at present provide semiquantitative information.

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