Rabbit systemic glucose metabolism map by total-body dynamic PET/CT technology
Haochen Wang\textsuperscript{a}, Xue Xie\textsuperscript{b}, Yanhua Duan\textsuperscript{c}, Leiyong Chai\textsuperscript{c}, Kun Li\textsuperscript{c}, Jianfeng Qiu\textsuperscript{b} and Zhaoping Cheng\textsuperscript{c}

Background This study evaluated total-body glucose metabolism in a preclinical lab animal, the rabbit, by employing a dynamic glucose metabolic image obtained with total-body fluorine-18 fluorodeoxyglucose (\textsuperscript{18}F-FDG) PET/computed tomography (PET/CT).

Methods The dynamic total-body PET/CT system was used to obtain glucose metabolic imaging from 10 sedated body-matched rabbits. The standard uptake value (SUV) of \textsuperscript{18}F-FDG was used to evaluate glucose metabolism. In addition, the correlation between glucose metabolism and sexes was assessed, as well as metabolic differences between left- and right sides.

Results We found significant distribution heterogeneity of glucose in several organs across the entire body. There were no significant metabolic differences between sexes and between bilateral sides in the 10 rabbits. Thereafter, we assayed the major organ SUV changes by dynamic PET/CT of the major organs. The heart, liver, and urinary system showed more \textsuperscript{18}F-FDG, whereas the skeletal muscle, brain, spinal cord, and lungs incorporated less \textsuperscript{18}F-FDG. The phenotype of \textsuperscript{18}F-FDG uptake was highly correlated with the physiological functions. The \textsuperscript{18}F-FDG accumulation in urinary system were observed which could reflect the renal parenchyma glucose metabolism indirectly. However, the low \textsuperscript{18}F-FDG uptake in the brain and spinal cord was due to sedation.

Conclusion The total-body glucose metabolic atlas depicted with \textsuperscript{18}F-FDG dynamic PET/CT may be used as a reference for assessing pathological \textsuperscript{18}F-FDG uptake. Furthermore, this study could be a reference for preclinical research involving abnormality of glucose metabolism.

Keywords: \textsuperscript{18}F-FDG, dynamic atlas, glucose metabolism, PET/CT, rabbit

Introduction Fluorine-18 fluorodeoxyglucose (\textsuperscript{18}F-FDG) PET has been widely used in clinical and preclinical research as a noninvasive examination approach for exploring animal physiology, biochemistry, and pharmacology in vivo. PET/computed tomography (CT) provides multiple classes of information that includes the body structure and molecular and metabolic changes [1]. Combined with different radioactive-labeled nuclides, PET/CT molecular imaging exhibits a wide range of functional imaging.

Glucose is the major carbon source for cellular biosynthesis and energy generation [2]. The imbalance of glycolytic rates in organs is always correlated with different metabolic intensities and oxygen uptake. For example, the brain, liver, and myocardium consistently take up more glucose and contain more mitochondria in the cytoplasm due to the high levels of their metabolism [3]. As a glucose analogue, \textsuperscript{18}F labeled FDG plays a key role in the study of systemic multi-organ metabolism. In PET imaging, differences detected by \textsuperscript{18}F-FDG may reflect responses to cellular energy consumption.

Before total-body dynamic PET/CT, the clinical usage of PET/CT was generally limited to routine static imaging and partial detector coverage, thus limiting its range of use [4]. Recently, the use of total-body PET systems has enabled whole-body investigations in lab animals for establishing lab animal metabolic profiles. In the daily clinical procedure at our center, patients need to remain stationary for 60 min after injecting \textsuperscript{18}F-FDG in dynamic and convenient PET until the distribution of the radio-labeled nuclide reaches equilibrium, before performing scanning. In this study, the total-body dynamic PET/CT scanner was used to obtain the rabbit glucose metabolic atlas of the major organs across the entire body and the distribution profile of the nuclide following intravenous injection to equilibrium.
Lab animals play a vital role in preclinical research. Total-body glucose metabolism has been mentioned in several animal models such as rats, dogs, and pigs [1,3,5,6]. None of the studies have examined rabbit glucose metabolism by total-body dynamic PET/CT. The aim of this work was, firstly, to evaluate the physiological glucose metabolism and to determine the normal range of SUV in New Zealand white rabbit for referencing. Secondly, the work established a new scheme to assess dynamic glycolytic rates of the major organs with 18F-FDG PET/CT.

**Methods**

**Animals**

Seven male and three female healthy New Zealand white rabbits were used in this study. All of the rabbits were 6 months old, and the body weights (BW) ranged from 2.15 kg to 3.55 kg (Table 1). Rabbits were acclimated in the facility for 1 week before the study. Every animal was fasted for 6 h, with free access to drinking water before scanning. The rabbits were sedated by inhaled isoflurane.

After the rabbits were sedated, we obtained a general physiological profile: BW, body length (from the vertex to the beginning of the tail) (Table 1). An indwelling needle was placed in the ear marginal vein of the rabbits, then the blood samples were obtained from the ear marginal vein via the indwelling needle. After that, the indwelling needle was rinsed with a small amount of normal saline. The study protocol was approved by the Shandong First Medical University Ethics Committee.

**PET/CT scanning**

PET/CT scanning was performed using the uEXPLORER (United Imaging Healthcare, Shanghai, China) after an intravenous injection of 18F-FDG. The tracer 18F-FDG was injected in the marginal ear vein in the scanning bed. The dose of 18F-FDG is 0.3 µCi/Kg. After injection, the dynamic total-body PET/CT scan was performed with the uEXPLORER for 3600s. All rabbits were fastened in a panel and placed in the prone position on the scanning bed.

The PET images were reconstructed using 3000s-3600s data, time of flight and point spread function modeling, two iterations and 20 subsets, matrix = 192 × 192, slice thickness = 2.89 mm, and pixel size = 3.125 × 3.125 × 2.886 mm³ with a Gaussian filter (FWHM = 3 mm). All necessary corrections including attenuation and scatter correction were performed.

**Table 1** Physiological profile of the New Zealand white rabbits

| No | Sex (M/F) | Weight (kg) | Length (mm) | Blood glucose (mmol/L) |
|----|-----------|-------------|-------------|------------------------|
| 1  | M         | 3.55        | 460.5       | 5.77                   |
| 2  | M         | 3.51        | 455.6       | 4.11                   |
| 3  | M         | 3.00        | 436.5       | 4.54                   |
| 4  | M         | 3.54        | 438.2       | 6.01                   |
| 5  | M         | 3.18        | 437.5       | 5.59                   |
| 6  | M         | 3.46        | 437         | 5.88                   |
| 7  | M         | 2.97        | 408         | 5.10                   |
| 8  | F         | 3.34        | 445.5       | 5.23                   |
| 9  | F         | 3.43        | 454.2       | 5.98                   |
| 10 | F         | 2.10        | 416.9       | 5.56                   |

F, female; M, male.

**Table 2** Results of lab test

| Rabbit No | ALT (U/L) | AST (U/L) | ALB (g/L) | Tbil. (mg/dL) | B. Crea. (µmol/L) | K⁺ (mmol/L) | Na⁺ (mmol/L) |
|-----------|-----------|-----------|-----------|---------------|-------------------|-------------|--------------|
| 1         | 51        | 15.4      | 42.6      | 0.4           | 64.8              | 3.97        | 142          |
| 2         | 40.9      | 32.6      | 56        | 0.2           | 84                | 3.81        | 143          |
| 3         | 23.4      | 27        | 56.1      | 0.3           | 77                | 4.52        | 149          |
| 4         | 33.2      | 40        | 51        | 1.1           | 111               | 4.41        | 139          |
| 5         | 22.9      | 60        | 39.4      | 1.1           | 51                | 5.37        | 133          |
| 6         | 17        | 67.7      | 36        | 2.2           | 67                | 3.66        | 141          |
| 7         | 31        | 11        | 41        | 1.0           | 73                | 4.61        | 134          |
| 8         | 41.5      | 43        | 33        | 0.7           | 73                | 5.01        | 139          |
| 9         | 50.2      | 77        | 43        | 1.7           | 95                | 4.30        | 148          |
| 10        | 16.6      | 50.1      | 38.2      | 0.9           | 52                | 5.55        | 149          |

ALB, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; B. Crea., blood creatine; Tbil., total bilirubin.
The measurement of $^{18}$F-FDG

The volume of interest (VOI) of major organs, including those of the brain, heart, bilateral lungs, liver, bilateral kidneys, bladder, vertebra, and bilateral thighs, was drawn manually on the selected image plane, with the most intense $^{18}$F-FDG uptake in each organ site identified by one radiologist and one experienced technician. At the same time, the VOIs were drawn for two or three separate parts of one organ. Thereafter, the mean SUV was automatically determined from the area selected. Mean SUVs of $^{18}$F-FDG in these organs were calculated from the concentration of the radio-tracer normalized according to the injected dose and BW. The $^{18}$F-FDG decay was corrected automatically by the software.

Statistical methods

Data were presented as the mean ± SD. Statistical analysis was performed using GraphPad Prism 8 (GraphPad Software Inc., La Jolla, CA, USA).

Results

After the rabbits were sedated, we obtained a general physiological profile: BW, body length (from the vertex to the beginning of the tail), and tested blood samples from the ear marginal vein (Table 1). We tested the representative assay index that potentially reflected the impairment of liver cells and bile duct, kidney function, liver synthesis function, and homeostasis (Table 2).

To analytically describe the glucose metabolic atlas across the whole body, we measured and compared the SUVs of the major organs and sexes of the rabbits (Fig. 1). In order to focus on exploring the heterogeneity between the organs, we controlled the variables of all the rabbits, including age, BW, and species.

We reconstructed all of the rabbits total-body dynamic PET images. The total-body PET image of No. 5 and No. 7 rabbits were used as the model shape for male rabbit image, female rabbit image, and summation image, respectively. We then assigned the mean SUVs of the major organs to depict the major organs $^{18}$F-FDG distribution (Fig. 2). The scale bar indicates the intensity of color which is positive related with the mean SUV. In these images, most of the major organs, including the liver, brain, and spinal cord are all absorbed moderate to high density of $^{18}$F-FDG. However, the skeletal muscles in the lower limbs and most of the bilateral lungs showed very little $^{18}$F-FDG uptake. The excretion of $^{18}$F-FDG was quickly appeared in the fusion images.

The mean SUV between male rabbits and female rabbits were compared. No significant difference in the mean SUV of each organ was detected between different sexes ($P > 0.05$). Moreover, no significant difference in the mean SUV of bilateral organs was found in any of the rabbits. Due to the large volume and functional complexity, we drew 3 VOIs, that is, the VOIs of the left lobe, the right lobe, and the middle lobe of the liver. Subsequent analysis still revealed no significant differences between all of the rabbits.

In all organs, the SUV peak was observed in the heart, kidney, and bladder (Fig. 3). Notably, clustering of all the rabbits based on the peak SUV revealed how the $^{18}$F-FDG was distributed in organs across the entire body.

In this study, we used the total-body dynamic PET/CT to draw the mean SUV-time curve (Fig. 4). Except for the bladder, most organs reached a peak and arrived at the plateau stage within 60 s. Accordingly, we focused on the first 60 s to monitor the changes. We depict the curve up to 3000 s due to the characteristic curve changes observed in the bladder.

We took rabbit No. 1 as the representative sample to calculate the time-activity curve to 3000 s and reconstructed...
Glucose metabolic activities of major organs in all 10 rabbits. (a) Histogram of the mean SUV showing the distribution in all 10 rabbits. (b) Major organ comparison. The full line indicates the mean SUV of the 10 rabbits. The filled area indicates the SD. (c) Comparisons of the glucose metabolic profiles among the different sexes. (d) Comparisons of the glucose metabolic profiles between the different side. (e) Heat map of the peak SUV of the major organs across the whole body of all rabbits. Each column represents a single rabbit, and each row represents the peak SUV of an organ. Samples were clustered using hierarchical clustering.
the PET images from 0 s to 6 s, 300 s, 600 s, 1800 s and 3000 s (Fig. 5). Images with good quality showed the $^{18}$F-FDG transit from the marginal ear vein to the right ventricle and to the lung and then into the systemic circulation. These 5 frames dynamically describe the changes of glucose metabolism in organs of the whole body at different moments.

Discussion

The dynamic total-body PET/CT offers a highly sensitive and efficient framework for studying systemic glucose metabolism. Brain $^{18}$F-FDG uptake varies widely because of the high rate of glucose utilization by neurons in humans. In our study, the measured SUV of $^{18}$F-FDG in rabbit brain was lower than sober human brain $^{18}$F-FDG uptake, which has been reported in the past, but similar to the results obtained in other lab animal studies [7]. The lower SUV in the rabbit brain was seemingly due to the suppression of brain activity by sedative [7,8]. We observed the same phenomenon in the spinal cord, where the absorption of $^{18}$F-FDG was the most similar to that in the brain, with a lower uptake rate and a lower peak value than most organs. Previous studies have found that $^{18}$F-FDG uptake in the spinal cord is a heterogeneous function of age, sex, and degree of functional impairment in humans. Furthermore, in pediatric populations, $^{18}$F-FDG uptake of the spinal cord was correlated with BW [9–11].

The liver is an essential multifunctional organ in mammals. Several previous studies have reported that BW and BMI are significant factors in the physiological $^{18}$F-FDG uptake of the liver [12]. Despite varying chiefly in both size and shape, the BW and BMI were controlled within a small range, in order to keep the consistency of this study. The results showed that transaminase and total bilirubin, indicators of impairments of liver cells and biliary tract cells, fell within the normal range. As a result, there was no significant difference in the liver uptake rate and SUV peak of the liver in each rabbit. Liver is a dual blood supply organ, making the $^{18}$F-FDG metabolism and uptake complex [13,14]. Meanwhile, previous studies found that the usage of anesthetized could cause an increase of $^{18}$F-FDG uptake in the liver [15]. This is mainly because the catabolism of anesthetic occurs in the liver, thereby increasing energy consumption. One shortage should be mentioned. Many research reported that the $^{18}$F-FDG phosphorylation in liver is not sufficient. It is about 25% [16] at 60 min. But it is generally low and relatively stable over a long time (at least 3 h after $^{18}$F-FDG injection) [17].

The lungs seem to have the second lowest SUV when compared with other organs that we analyzed. That is mainly because lung is hollow organ which contains air. A previous study showed that $^{18}$F-FDG was slightly detectable in the normal lung, whereas in lung tumor and acute lung injury, the SUV is expected to be elevated due to the high metabolic activity of tumor cells and neutrophils.

The mean SUV–time dynamic curves of the major organs which reflect the blood pool change and $^{18}$F-FDG distribution in the 10 rabbits.
present in the lung [5,18,19]. It is conceivable that the high SUV peak of the lung during the first minute is mainly due to the first pass of high $^{18}$F-FDG concentration in the blood pool. After the first $^{18}$F-FDG pass in the blood, the pulmonary concentration of $^{18}$F-FDG dropped quickly to the level of the spine and brain (Fig. 4).

The bilateral thighs took up little $^{18}$F-FDG in all the rabbits examined in our study. This appears to be due to the lack of skeletal muscle movement, as well as the use of both glucose and fatty acids as energy sources in muscle tissues [20]. By contrast, the myocardium showed more and earlier uptake of $^{18}$F-FDG in the dynamic images. However, individual variation was significant. On the contrary, the FDG uptake of myocardium is also related to anesthesia. Anesthesia can affect FDG uptake by changing regional blood flow, heart rate, and cardiac pre- and post-load [5]. Variability of $^{18}$F-FDG uptake in the myocardium has been reported in humans [21,22]. Importantly, cardiac movement makes assessments challenging for manually drawing VOIs.

We accurately measured the renal parenchymal SUV and avoided contamination from other parts of the renal pelvis. In the initial $^{18}$F-FDG injection stage, the kidneys absorbed the $^{18}$F-FDG quickly with the peak SUV and mean SUV higher than that of most organs other than the lungs and heart. After about 300s, the $^{18}$F-FDG began to accumulate in each rabbit bladder. The $^{18}$F-FDG accumulation in urinary system is mostly because of the urine excretion. Even in renal parenchymal, the SUV change cannot totally reflect the glucose metabolism. The clearance of $^{18}$F-FDG depends substantially on the glomerular filtration rate. In the remaining scanning period, the SUV gradually increased in urinary system; however, the accumulation and uptake rate varied due to food...
deprivation prior to scanning. The hydration of each subject varied significantly. As the previous study reported, $^{18}$F-FDG accumulation was significantly reduced in the bladder when preconditioned with urethral catheterization, followed by hydration or intravenous furosemide before $^{18}$F-FDG-PET analysis [23,24].

Lipid metabolism, especially the visceral adipose tissue is critical for glucose metabolism. The excess visceral fat is related to abnormal glucose tolerance. In addition, Kuhla et al. [25] reported that the ApoE can also effect glucose metabolism in brain. In this study all the animals are controlled the variables. The age of New Zealand white rabbit in our study is controlled at about 6-month old. It is the transition phase from juvenile stage to sexual maturity stage. In this stage, the visceral adipose content is quite low. Therefore, the assessment of visceral adipose tissue is limited. The influencing factors of visceral adipose tissue on glucose metabolism should be considered on animal models of diabetes or aging.

Notably, several limitations are pertinent to the study. (1)This work is the affected by sedation. There may be some discrepancies in the organ metabolism when the subjects are sober. Despite the uniform in species, age, BW and treatment of the rabbits, there was a high variability in the uptake of $^{18}$F-FDG, which may mirror the individual variation. (2) The organ heterogeneity in glucose metabolism was reported in many studies. One unified scanning strategy and preconditioning approach is not suitable for different animal model or different target organ. (3)The sample size is not quite enough. In future studies, researchers could vary the scanning and preconditioning procedure according to different experimental purposes.

We have demonstrated that it is possible to generate total body glucose metabolism map by dynamic whole-body PET/CT in rabbit. There are numerous contexts in which this model could be clinically relevant. For example, these models provide a reference standard that could be used for the interpretation of preclinical studies in older patients or those with cancer or deregulated glucose metabolism. The distribution and normal range of SUV in normal organs identified in this study reflect the glucose metabolism which provides a reference for future dynamic PET imaging and research.

**Acknowledgements**

This work was supported by the Jinan Science and Technology Foundation [Grant No. 2020GXRC018], the Academic Promotion Program of Shandong First Medical University [Grant No. 2019QL009] and [Grant No. 2019LJ005], and the Taishan Scholars Program of Shandong Province [Grant No. TS201712065].

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Fletcher JW, Djulbegovic B, Soares HF, Siegel BA, Lowe VJ, Lyman GH, et al. Recommendations on the use of $^{18}$F-FDG PET in oncology. J Nucl Med 2008; 49:480–508.
2. Baralle F, Plancheij J, Dentin R, Gulmeau S, Postic C. Integration of ChREBP-mediated glucose sensing into whole body metabolism. Physiology (Bethesda) 2015; 30:428–437.
3. Jang C, Hui S, Zeng X, Cowan AJ, Wang L, Chen Li, et al. Metabolite exchange between mammalian organs quantified in pigs. Cell Metabol 2019; 30:594–605.e3.
4. Cherry SR, Badawi RD, Karp JS, Moses WW, Price P, Jones T. Total-body imaging: transforming the role of positron emission tomography. Sci Transl Med 2017; 9eaal6169.
5. Lee MS, Lee AR, Jung MA, Lee HH, Choi J-H, Chung H-W, et al. Characterization of physiologic $^{18}$F-FDG uptake with PET/CT in dogs. Vet Radiol Ultrasound 2010; 51:670–673.
6. Min W, Fang P, Huang G, Shi M, Zhang Z. The decline of whole-body glucose metabolism in ovariectomized rats. Exp Gerontol 2018; 113:106–112.
7. Aistrup AK, Smith DF. Anesthesia for positron emission tomography scanning of animal brains. Lab Anim 2013; 47:12–18.
8. Shamas A, Lim R, Charron M. Pediatric PET/CT: physiologic uptake, normal variants, and benign conditions. Radiographics 2009; 29:1487–1486.
9. Amin A, Rosenbaum SJ, Bockisch A. Physiological $^{18}$F-FDG uptake by the spinal cord: is it a point of consideration for cancer patients. J Neurooncol 2012; 107:609–615.
10. Taralli S, Lecissoti L, Mattoli WV, Castaldi P, de Waure C, Mancuso A, et al. Physiological activity of spinal cord in children, an $^{18}$F-FDG PET-CT study. Spine (Phila Pa 1976) 2015; 40:E647–E652.
11. Aiello M, Alfano V, Salvatore E, Caleavale G, Picardi M, Della Pepa R, et al. [(18)F]FDG uptake of the normal spinal cord in PET/CT imaging: comparison with PET/CT imaging. EJNMMI Res 2020; 10:91.
12. Keiding S, Sorensen M, Frisch K, Gommesen LC, Munk OL. Quantitative of liver functions. Am J Nucl Med Mol Imaging 2018; 8:73–85.
13. Keiding S. Bringing physiology into PET of the liver. J Nucl Med 2012; 53:425–433.
14. Brixi G, Ziegler SI, Bellermann ME, Doll J, Schossier R, Lucht R, et al. Quantification of [(18)F]FDG uptake in the normal liver using dynamic PET: impact and modeling of the dual hepatic blood supply. J Nucl Med 2001; 42:1265–1273.
15. Lee KH, Ko BH, Paik JY, Jung KH, Cho YeS, Choi Y, et al. Effects of anesthetic agents and fasting duration on $^{18}$F-FDG biodistribution and insulin levels in tumor-bearing mice. J Nucl Med 2005; 46:1531–1536.
16. Keramida G, Anagnostopoulous CD, Peters AM. The extent to which standardized uptake values reflect FDG phosphorylation in the liver and spleen as functions of time after injection of $^{18}$F-fluorodeoxyglucose. EJNMMI Res 2017; 7:13.
17. Seith F, Schmidt H, Kunz J, Kistner T, Gatids S, Nikolaou K, et al. Simulation of tracer dose reduction in $^{18}$F-FDG PET/MRI: effects on oncologic readers, image quality, and artifacts. J Nucl Med 2017; 58:1699–1705.
18. Capitanio S, Nardin J, Noriani AR, Rossetti C. PET/CT in neunological lung diseases: current applications and future perspectives. Eur Respir Rev 2016; 25:247–258.
19. Vansteenkiste JF, Stroobants SG, Dupont PJ, De Leen PR, Verbeeken EK, Denefle GJ, et al. Pronostic importance of the standardized uptake value on $^{18}$F-fluoro-2-deoxy-glucose-positron emission tomography scan in non-small-cell lung cancer: an analysis of 125 cases. Leuven Lung Cancer Group. J Clin Oncol 1999; 17:3201–3206.
20. Engel H, Steiner H, Buck A, Berthold T, Huch Bnna RA, von Schultess GK. Whole-body PET: physiological and artificial fluorodeoxyglucose accumulations. J Nucl Med 1996; 37:441–446.
21. Ramos CD, Erdi YE, Gonen M, Riedel E, Yeung HW, Macapinlac HA, et al. FDG-PET standardized uptake values in normal anatomical structures using iterative reconstruction segmented attenuation correction and filtered back-projection. Eur J Nucl Med 2001; 28:155–164.
22. Tan LT, Ong KL. Semi-quantitative measurements of normal organs with variable metabolic activity on PET/CT imaging. Ann Acad Med Singap 2004; 33:183–185.
23. Kosuda S, Fisher S, Wahl RL. Animal studies on the reduction and/or dilution of 2-deoxy-2-$^{18}$Ffluoro-D-glucose (FDG) activity in the urinary system. Ann Nucl Med 1997; 11:213–218.
24. Cussó L, Desco M. Suppression of $^{18}$F-FDG signal in the bladder on small animal PET-CT. PLoS One 2018; 13:e0205610.
25. Kuhla A, Meuth L, Stenzel J, Lindner T, Lappe C, Kurth J, et al. Longitudinal [(18)F]FDG-PET/CT analysis of the glucose metabolism in ApoE-deficient mice. EJNMMI Res 2020; 10:119.