Physiol. Res. 55: 653-659, 2006

Assessment of Brown Adipose Tissue Activity in Rats by ^{99m}Tc-Sestamibi Uptake

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Received October 31, 2005
Accepted January 25, 2006
On-line available February 23, 2006

Summary

Brown adipose tissue (BAT) physiology and imaging have recently attracted considerable attention. BAT is characterized both by enhanced perfusion and increased mitochondrial activity. ^{99m}Tc-sestamibi is a lipophilic cationic tracer that concentrates in mitochondria. Data on the accumulation of ^{99m}Tc-sestamibi in BAT are currently lacking. This study investigates the *in vivo* ^{99m}Tc-sestamibi uptake in rat BAT. ^{99m}Tc-sestamibi was administered in male Wistar rats of various age and body size. ^{99m}Tc-sestamibi uptake was measured *in vitro* in BAT and white fat (WF) together with cytochrome *c* oxidase activity. Both ^{99m}Tc-sestamibi uptake and cytochrome *c* oxidase activity were higher in BAT than in WF ($P<0.05$). ^{99m}Tc-Sestamibi uptake in both BAT and WF was negatively related to body weight ($r = -0.96$ and -0.89, respectively) as was the BAT/WF uptake ratio ($r = -0.85$). These data show a higher ^{99m}Tc-sestamibi uptake in BAT compared to WF, in agreement with the high mitochondrial content and respiratory activity of the former. The strong negative correlation between ^{99m}Tc-sestamibi uptake in BAT and body weight (negative allometry), is in accordance to increased needs of thermogenesis in smaller animals. Implications of increased ^{99m}Tc-sestamibi uptake in BAT in radionuclide imaging are also discussed.

Key words

Brown adipose tissue • ^{99m}Tc-sestamibi • Mitochondria • Allometry • Imaging

Introduction

Brown adipose tissue (BAT) is found mainly in small and hibernating mammals, especially in neonates. It is characterized by the unique ability of non-shivering thermogenesis mediated by the mitochondrial uncoupling protein (UCP1), or thermogenin, which uncouples the electron transport chain from oxidative phosphorylation (Lindberg *et al.* 1967, Nelson and Cox 2004). BAT function is under close, multilevel control by the sympathetic nervous system. Exposure to cold promotes lipolysis *via* β-adrenergic stimulation, thus resulting in increased fatty acid oxidation and thermogenesis. BAT may also play a role in the pathogenesis of obesity (Cannon and Nedergaard 2004). Histologically, BAT is characterized by abundant sympathetic innervation, rich

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vascularization – necessary for heat convection to the bloodstream – and an extraordinarily high number of mitochondria (Cinti 2001).

Although metabolic imaging has traditionally been the cornerstone of nuclear medicine, it was not until recently that a number of studies appeared reporting on the importance of BAT imaging in experimental and clinical settings (Weber 2004). Okuyama *et al*. (2002) have observed that radiolabeled metaiodobenzyl-guanidine (MIBG) accumulation in BAT was higher than in white fat (WF) and comparable to that in the heart of rats. The same authors showed a non-tumoral radio-pharmaceutical accumulation in the nape in ^{123}I-MIBG scans of children with neuroblastoma, which was attributed to tracer uptake in BAT (Okuyama *et al.* 2003). Fukuchi *et al.* (2003), analyzing pediatric cardiac perfusion scans with ^{99m}Tc-tetrofosmin, reported intrascapular radiotracer accumulation compatible with BAT in 17 % of infants and children examined, which was more intense during the winter season. The presence of BAT-compatible ^{18}F-fluorodeoxyglucose (^{18}F-FDG) accumulation in the supraclavicular, neck or even mediastinal region during PET imaging has also been repeatedly shown (Hany *et al.* 2002, Cohade *et al.* 2003a,b, Yeung *et al.* 2003, Truong *et al.* 2004).

^{99m}Tc-sestamibi is the first clinically introduced alternative to ^{201}Tl for myocardial perfusion scintigraphy, and has also found extensive application in parathyroid and tumor imaging. Tissue uptake of ^{99m}Tc-sestamibi is proportional to regional blood flow, and the tracer is retained within the mitochondria (Bernard *et al.* 1998). Thus, ^{99m}Tc-sestamibi seems to be a good candidate tracer for BAT imaging. Moreover, the field of view during ^{99m}Tc-sestamibi scintigraphy for myocardial or parathyroid imaging is a favorable one, including the neck and supraclavicular area where BAT is located. With the exception of a recently published clinical case report (Higuchi *et al.* 2004), clinical or experimental data regarding ^{99m}Tc-sestamibi uptake in BAT are lacking. Thus, the aim of the present study was to explore ^{99m}Tc-sestamibi uptake in rat BAT in relation to mitochondrial activity and thermoregulatory aspects.

Methods

Animals

Eight male Wistar rats, aged 25 days to 8 months and weighing 40 to 450 g, were enrolled in the study. Rats were individually housed in cages except for the smallest one, which was housed with its mother. The animals lived under a 12-hour light/dark cycle with controlled temperature (23-25 °C) and humidity (50-70 %). Commercial rat chow and tap water were provided *ad libitum*. The project was approved by the appropriate state authority. All procedures were in accordance with the *European Union Guidelines for the Care and Use of Laboratory Animals*, as well as the *Principles of Laboratory Animal Care* (NIH publication No. 86-23, revised 1985).

Radiopharmaceutical

Tetrakis (2-methoxy isobutyl isonitrile) (Cardiolite®) was purchased from Bristol-Myers Squibb Medical Imaging, Belgium. Labeling was performed according to the manufacturer's instructions by adding 50 mCi (1850 MBq) of freshly eluted ^{99m}Tc (as pertechnetate) in a vial containing 1 mg of Cardiolite, to a final volume of 2.5 ml saline. Radiochemical purity has been previously validated in our department (GA, SG, CK) and has been consistently found to be over 95 %.

Surgical procedures and ^{99m}Tc-sestamibi administration

Under anesthesia with 10 mg/kg body weight xylazine and 50 mg/kg body weight ketamine hydrochloride injected intraperitoneally, the skin of the neck region was shaved and a small incision was made on the right side of the neck, disclosing the internal jugular vein. All animals were studied using a single vial of the tracer. The radiopharmaceutical was administered through the vein by using insulin syringes with a 29G x ½-inch permanently attached needle (BD, USA), with the neck in an overextended position under a magnifying lamp. The dose administered was 0.01 mg of Cardiolite per kg body weight, corresponding to 500 μCi (18.5 MBq) ^{99m}Tc-sestamibi one hour after labeling. The radio-pharmaceutical was administered within 4 hours of preparation, and syringes were weighed before and after injection on a four-digit electronic balance. The injected volume was adjusted to 0.1-0.3 ml by diluting with normal saline, and administration was performed at such a rate that no distention of the jugular vein was produced. After administration, gentle pressure was applied on the vessel for one minute by means of a wet piece of cotton, which was saved for later activity measurement. A standard was prepared by diluting an extra dose of ^{99m}Tc-sestamibi in 500 ml of distilled water and then collecting two samples of 1 ml by precision pipetting.

Tissue harvesting

During the two hours following ^{99m}Tc-sestamibi injection, the animals lay unconscious in the supine position on the surgery table. To maintain their body temperature, rats were placed in the proximity of a diffuse heat source and were covered with a "blanket" made of aluminum foil. Anesthesia was maintained by administering approximately 10 % of the initial dose, when necessary. Two hours after tracer injection the animals were sacrificed by exsanguination through transthoracic cardiac puncture, and tissue harvesting was completed within 10-15 min. WF was collected from intra-abdominal sites. BAT collected from interscapular region was easily identified by its unique location between the scapulae and the characteristic brownish color (Rothwell and Stock 1985).

Tissues were dissected free of connective tissue, cleaned with saline, blotted on absorbent paper, and weighed. A small piece from each tissue was then sequestered, weighed, snap-frozen in liquid nitrogen, and stored at –80 °C for subsequent biochemical analysis. To check for potential extravasation of the radiopharmaceutical during injection, tissue blocks of approximately equal size including part of the jugular vein and surrounding tissues were bilaterally removed, weighed and measured for radioactivity.

Measurement of ^{99m}Tc-sestamibi uptake

The radioactivity of tissues and the standard was measured in a well-type γ-counter (Oxford Instruments, USA) for 10 min. Measurements were corrected for the physical decay of ^{99m}Tc ($T_{1/2}$ = 6.02 h). ^{99m}Tc-sestamibi uptake (U) as a percentage of the injected dose (ID) per gram of tissue was calculated as follows:

$$U\ (\%ID/g) = \frac{C_t}{C_{st}} \cdot \frac{W_{st}\ (mg)}{W_t\ (mg)} \cdot \frac{200}{WID_{net}\ (mg)}$$

where: C_t, tissue counts; C_{st}, counts of 1 ml of standard; W_{st}, weight of the standard, calculated as the weight difference between the full and empty syringe; W_t, weight of tissue; 200, a constant valid for a standard volume of 500 ml and a standard sample volume of 1 ml; WID_{net}, net weight of the injected dose. WID_{net} was calculated as follows:

$$WID_{net}\ (mg) = WID\ (mg) - \left(W_{st}(mg) \cdot \frac{C_{\text{cot}} + C_{inj} - C_{contr} \cdot \frac{W_{inj}\ (mg)}{W_{contr}\ (mg)}}{C_{st}} \right)$$

where: WID, weight of injected dose, calculated as the weight difference between the full and empty syringe; C_{cot}, counts of the piece of cotton used to compress the jugular vein; C_{inj}, counts of tissue block on the side of the injection; C_{contr}, counts of tissue block on the contralateral side; W_{inj}, weight of tissue block on the side of injection; W_{contr}, weight of tissue block on the contralateral side.

Table 1. Original and derived data of the experimental animals

Rat #	Body weight g	BAT uptake %ID/g	WF uptake %ID/g	BAT/WF uptake ratio	BAT CCO U/g	WF CCO U/g
1	40	4.74	0.64	7.35	27.40	2.00
2	139	1.20	0.30	4.05	21.60	3.60
3	153	0.83	0.14	5.82	17.70	4.60
4	206	0.58	0.14	4.05	25.00	4.00
5	224	0.45	0.13	3.47	35.20	4.60
6	267	0.48	0.15	3.30	22.60	4.00
7	284	0.47	0.13	3.63	20.10	4.00
8	450	0.09	0.02	3.77	28.20	3.20

BAT, brown adipose tissue; ID, injected dose; WF, white fat; CCO, cytochrome-c oxidase; U/g, units per gram

Biochemical analysis

Cytochrome c oxidase (CCO) of BAT and WF was assayed spectrophotometrically as an index of the mitochondrial content of the tissues, according to an assay kit from Sigma (St. Louis, MO). CCO activity was determined by monitoring the oxidation of ferrocytochrome c to ferricytochrome c at 550 nm and 25 °C for 45 s (Storrie and Madden 1990). Ten mg of tissue were homogenized in 99 volumes of 10 mM Tris-HCl, 0.25 M sucrose, pH 7.3, at 0 °C. Five µl (in the case of BAT) or 30 µl (in the case of white fat) of the homogenate were mixed with 570 µl of an assay buffer containing 10 mM Tris-HCl, 120 mM KCl, pH 7.0, and the volume was brought to 630 µl with homogenization buffer. Then 30 µl of 0.22 mM ferrocytochrome c (reduced with sodium hydrosulfite) were added to start the reaction. CCO activity was expressed as U/g wet tissue, 1 U corresponding to 1 µmol of ferrocytochrome c oxidized per min. The assay was performed on a single day to eliminate inter-assay variability. CCO levels were measured in duplicate for all specimens. The intra-assay coefficient of variation was 9 %.

Statistics

Paired comparisons were performed using Wilcoxon's test. Association between variables was

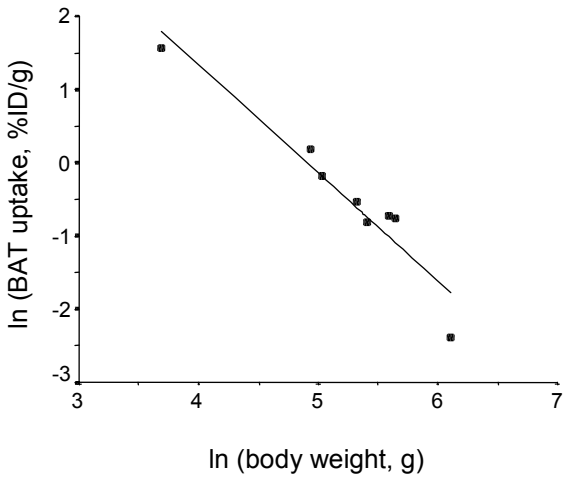

Fig. 1. Relationship between ^{99m}Tc-sestamibi uptake in BAT and body weight after logarithmic data transformation. ln (BAT uptake) = 7.24 – 1.48 ln (body weight), r = –0.96, P<0.001.

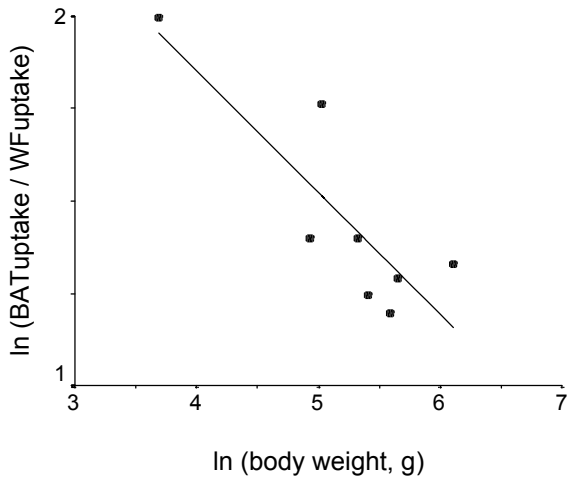

Fig. 2. Relationship between ^{99m}Tc-sestamibi BAT / WF uptake ratio and body weight after logarithmic data transformation. ln (BAT/WF uptake) = 3.17 – 0.33 ln (body weight), r = –0.85, P<0.01.

assessed by means of linear regression analysis. The level of statistical significance was set at P<0.05.

Results

Detailed experimental results are presented in Table 1. A wide range of ^{99m}Tc-Sestamibi uptake values is evident in both BAT and WF and an inverse relation to animal body size was also demonstrated. BAT exhibits a significantly higher uptake and CCO activity than WF (P<0.05). The ^{99m}Tc-sestamibi BAT/WF uptake ratio was 4.4±1.4. The BAT/WF CCO activity ratio was 7.0±3.1. ^{99m}Tc-sestamibi uptake in BAT showed a strong negative relation with body weight, best modeled by linear regression after logarithmic transformation of the data (Fig. 1). ^{99m}Tc-sestamibi uptake in WF also showed a negative relationship with body weight [ln (uptake) = 4.07 – 1.15 ln (body weight), r = –0.89, P<0.01]. However, the decline of uptake with increasing body size, was less sharp compared to that of BAT (b, 1.15 vs 1.48, P<0.05). More important, the BAT/WF uptake ratio declined with increasing body weight (Fig. 2).

Discussion

To our knowledge, data on ^{99m}Tc-sestamibi uptake in BAT were currently not available. Thus, the aim of this study was to investigate ^{99m}Tc-sestamibi uptake in rat BAT and relate it to mitochondrial activity and animal size. We found that ^{99m}Tc-sestamibi uptake in BAT was higher compared to WF and highly dependent on body size.

Environmental temperature is important when studying BAT physiology, and the role of hypothermia during sedation or anesthesia has recently been stressed (Weber 2004). Acute exposure to cold has been shown to greatly increase ^{18}F-FDG uptake in BAT of rats (Tatsumi *et al.* 2004). BAT imaging by either ^{123}I-MIBG or ^{99m}Tc-tetrofosmin has been observed more frequently during the winter season in children (Okuyama *et al.* 2002, Fukuchi *et al.* 2003). In the present study, rats were housed at 23-25 °C, a temperature slightly lower than the lower critical temperature for small rodents (27.5-29 °C). However, no appreciable metabolic stimulation has been shown in small rodents at 23-25 °C (Gordon 1988, Golozoubova *et al.* 2004).

Ketamine used for anesthesia, may exert a certain degree of sympathomimetic activity (Marshal and Longnecker 1992). The dose of ketamine we used (50 mg/kg body weight) was rather low compared to the usually recommended ketamine/xylazine combination containing 75-100 mg per kg body weight (Sharp and La Regina 1998, Waynforth and Flecknell 1992). Although Tatsumi *et al.* (2004) have reported a catecholamine-mediated increase in ^{18}F-FDG uptake in BAT of Lewis rats at a dose as low as 30 mg/kg body weight, the validity of this observation in other rat strains has not been confirmed.

Regarding the optimal time for tissue harvesting, based on findings on ^{99m}Tc-sestamibi kinetics in the rat heart and blood (Onoguchi *et al.* 2003), samples were collected two hours after the injection, assuming that

radiotracer uptake in BAT would be stable by that time.

The BAT/WF CCO activity ratio was seven, probably reflecting the mitochondrial abundance in BAT. As ^{99m}Tc-sestamibi is concentrated mainly within mitochondria according to transmembrane potentials (Piwnica-Worms *et al.* 1990, Chiu *et al.* 1990, Carvalho *et al.* 1992), a similar ^{99m}Tc-sestamibi BAT/WF uptake ratio would have been expected. However, ^{99m}Tc-sestamibi uptake was only four times higher in BAT than in WF. A possible explanation for this discrepancy could be the reduced transmembrane potential in BAT mitochondria, as a consequence of proton influx through UCP1. Increased lipid content in WF compared to BAT (80 % vs 30 %) (Lunati *et al.* 1999) could also contribute, given the lipophilicity of ^{99m}Tc-sestamibi.

^{99m}Tc-sestamibi uptake in BAT was highly dependent on animal body size. The uptake-body weight relationship was modeled by the equation $Y = aX^b$, (Y, uptake; X, body weight; a, proportionality constant; b, scaling exponent), linearized to $\ln Y = \ln a + b \ln X$ by logarithmic transformation. Equations of this type, called allometric (as contrasted to isometric), are common in studies correlating physiologic or metabolic parameters to body size. Figure 1 shows that ^{99m}Tc-sestamibi uptake in BAT declines as animal size (represented by body weight) increases (negative allometry). A similar inverse relationship between ^{99m}Tc-sestamibi uptake in WF and animal body size was found, but with a lower scaling factor (b). Importantly, the BAT/WF uptake ratio also declines with increasing body weight, showing the importance of BAT activity (and hence active thermogenesis) in small-sized animals. As body surface area (BSA) changes in a non-linear fashion (disproportionately) with respect to body weight (Schmidt-Nielsen 1984), the relatively increased BSA (measure of heat loss) in relation to body weight (measure of metabolic heat production) in smaller animals requires more effective thermogenesis. Increased ^{99m}Tc-sestamibi uptake per unit of tissue mass in the BAT of smaller as compared to larger rats may be associated with increased thermogenic efficacy in the former.

The literature is not conclusive regarding the relative contribution of perfusion and mitochondrial activity to ^{99m}Tc-sestamibi uptake in tissues. In a recent case report of a patient with positive BAT imaging in the neck using ^{18}F-FDG PET/CT, Higuchi *et al.* (2004) have provided some interesting data. When a ^{201}Tl/^{99m}Tc-sestamibi dual-tracer SPECT was performed, the region corresponding to the PET/CT findings showed intense ^{99m}Tc-sestamibi, but no ^{201}Tl (a "pure" perfusion tracer) uptake, suggesting a major role for mitochondrial activity in ^{99m}Tc-sestamibi uptake.

Conclusion

The present study shows that both ^{99m}Tc-sestamibi uptake and mitochondrial cytochrome c oxidase activity are significantly higher in BAT than in WF of rats. ^{99m}Tc-sestamibi uptake in BAT is related with body weight by a strong negative allometric relationship indicating increased BAT activity in smaller animals. The relative contribution of perfusion and mitochondrial activity to ^{99m}Tc-sestamibi uptake in BAT remains to be determined.

Acknowledgements

Authors wish to thank Dr. N. Kokolis, Prof. of Physiology, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, for warm and generous hospitality in his laboratory where a part of these experiments was conducted.

References

BERNARD BF, KRENNING EP, BREEMAN WAP, ENSING G, BENJAMINS H, BAKKER WH, VISSER TJ, DE JONG M: ^{99m}Tc-MIBI, ^{99m}Tc-Tetrofosmin and ^{99m}Tc-Q12 in vitro and in vivo. *Nucl Med Biol* **25**: 233-240, 1998.

CANNON B, NEDERGAARD J: Brown adipose tissue: function and physiological significance. *Physiol Rev* **84**: 277-359, 2004.

CARVALHO PA, CHIU ML, KRONAUGE JF, KAWAMURA M, JONES AG, HOLMAN BL, PIWNICA-WORMS D: Subcellular distribution and analysis of technetium-99m-MIBI in isolated perfused rat hearts. *J Nucl Med* **33**: 1516-1521, 1992.

CHIU ML, KRONAUGE JF, PIWNICA-WORMS D: Effect of mitochondrial and plasma membrane potentials on accumulation of hexakis (2-methoxyisobutylisonitrile) technetium(I) in cultured mouse fibroblasts. *J Nucl Med* **31**: 1646-1653, 1990.

CINTI S: The adipose organ: morphological perspectives of adipose tissues. *Proc Nutr Soc* **60**: 319-328, 2001.

COHADE C, MOURTZIKOS KA, WAHL RL: "USA-Fat": prevalence is related to ambient outdoor temperature – evaluation with ^{18}F-FDG PET/CT. *J Nucl Med* **44**: 1267-1270, 2003a.

COHADE C, OSMAN M, PANNU HK, WAHL RL: Uptake in supraclavicular area fat ("USA-Fat"): description on ^{18}F-FDG PET/CT. *J Nucl Med* **44**: 170-176, 2003b.

FUKUCHI K, ONO Y, NAKAHATA Y, OKADA Y, HAYASHIDA K, ISHIDA Y: Visualization of interscapular brown adipose tissue using ^{99m}Tc-tetrofosmin in pediatric patients. *J Nucl Med* **44**: 1582-1585, 2003.

GOLOZOUBOVA V, GULLBERG H, MATHIAS A, FORREST D, CANNON B, VENNSTOM B, NEDERGAARD J: Depressed thermogenesis but competent brown adipose tissue recruitment in mice devoid of all hormone-binding thyroid hormone receptors. *Mol Endocrinol* **18**: 384-401, 2004.

GORDON CJ: Simultaneous measurement of preferred ambient temperature and metabolism in rats. *Am J Physiol* **254**: R229-R234, 1988.

HANY TF, GHAREHPAPAGH E, KAMEL EM, BUCK A, HIMMS-HAGEN J, VON SCHULTHESS GK: Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. *Eur J Nucl Med Mol Imaging* **29**: 1393-1398, 2002.

HIGUCHI T, KINUYA S, TAKI J, NAKAJIMA K, IKEDA M, NAMURA M, TONAMI N: Brown adipose tissue: evaluation with ^{201}Tl and ^{99m}Tc-sestamibi dual-tracer SPECT. *Ann Nucl Med* **18**: 547-549, 2004.

LINDBERG O, DEPIERRE J, RYLANDER E, AFZELIUS BA: Studies of the mitochondrial energy-transfer system of brown adipose tissue. *J Cell Biol* **34**: 293-310, 1967.

LUNATI E, MARZOLA P, NICOLATO E, FEDRIGO M, VILLA M, SBARBATI A: In vivo quantitative lipidic map of brown adipose tissue by chemical shift imaging at 4.7 Tesla. *J Lipid Res* **40**: 1395-1400, 1999.

MARSHAL BE, LONGNECKER DE: General anesthetics. In: *The Pharmacological Basis of Therapeutics*. GOODMAN GILMAN A, RALL TW, NIES AS, TAYLOR P (eds), McGraw Hill, New York, 1992, pp 285-310.

NELSON DL, COX MM: *Lehninger Principles of Biochemistry*. Freeman, New York, 2004, pp 717-718.

OKUYAMA C, SAKANE N, YOSHIDA T, SHIMA K, KUROSAWA H, KUMAMOTO K, USHIJIMA Y, NISHIMURA T: ^{123}I- or ^{125}I-metaiodobenzylguanidine visualization of brown adipose tissue. *J Nucl Med* **43**: 1234-1240, 2002.

OKUYAMA C, USHIJIMA Y, KUBOTA T, YOSHIDA T, NAKAI T, KOBAYASHI K, NISHIMURA T: ^{123}I-metaiodobenzylguanidine uptake in the nape of the neck of children: likely visualization of brown adipose tissue. *J Nucl Med* **44**: 1421-1425, 2003.

ONOGUCHI M, TAKAYAMA T, MIYATI T, TONAMI N, SUMI Y, MAEHARA T: Comparison of ^{99m}Tc-MIBI uptakes on planar images with those in excised rat organs. *Nucl Med Commun* **24**: 47-54, 2003.

PIWNICA-WORMS D, KRONAUGE JF, CHIU ML: Uptake and retention of hexakis (2-methoxyisobutyl isonitrile) technetium(I) in cultured chick myocardial cells. Mitochondrial and plasma membrane potential dependence. *Circulation* **82**: 1826-1838, 1990.

ROTHWELL NJ, STOCK MJ: Biological distribution and significance of brown adipose tissue. *Comp Biochem Physiol A* **82**: 745-751, 1985.

SCHMIDT-NIELSEN K: *Scaling. Why is Animal Size so Important?* Cambridge University Press, Cambridge, 1984, pp 77-82.

SHARP PE, LA REGINA MC. *The Laboratory Rat*. CRC Press, Boca Raton, 1998, pp 105.

STORRIE B, MADDEN EA. Isolation of subcellular organelles. *Methods Enzymol* **182**: 203-225, 1990.

TATSUMI M, ENGLES JM, ISHIMORI T, NICELY O'B, COHADE C, WAHL RL: Intense ^{18}F-FDG uptake in brown fat can be reduced pharmacologically. *J Nucl Med* **45**: 1189-1193, 2004.

TRUONG MT, ERASMUS JJ, MUNDEN RF, MAROM EM, SABLOFF BS, GLADISH GW, PODOLOFF DA, MACAPINLAC HA: Focal FDG uptake in mediastinal brown fat mimicking malignancy: a potential pitfall resolved on PET/CT. *Am J Roentgenol* **183**: 1127-1132, 2004.

WAYNFORTH HB, FLECKNELL PA: *Experimental and Surgical Technique in the Rat.* Academic Press, London, 1992, pp 118-119.

WEBER WA: Brown adipose tissue and nuclear medicine imaging. *J Nucl Med* **45**: 1101-1103, 2004.

YEUNG HW, GREWAL RK, GONEN M, SCHODER H, LARSON SM: Patterns of ^{18}F-FDG uptake in adipose tissue and muscle: a potential source of false-positives for PET. *J Nucl Med* **44**: 1789-1796, 2003.

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