Animal Handling for *In Vivo* Imaging

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Society of Nuclear Medicine, Washington D.C.

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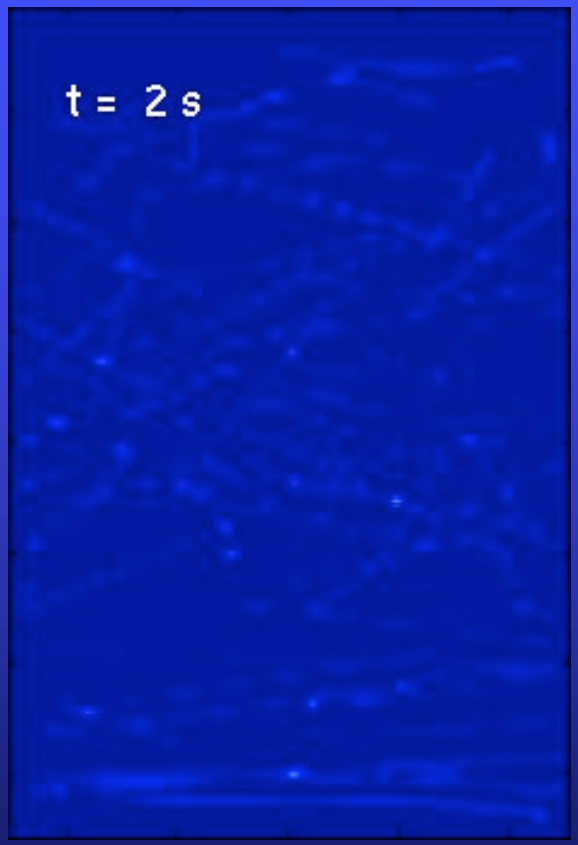
UCDAVIS
UNIVERSITY OF CALIFORNIA

cmg

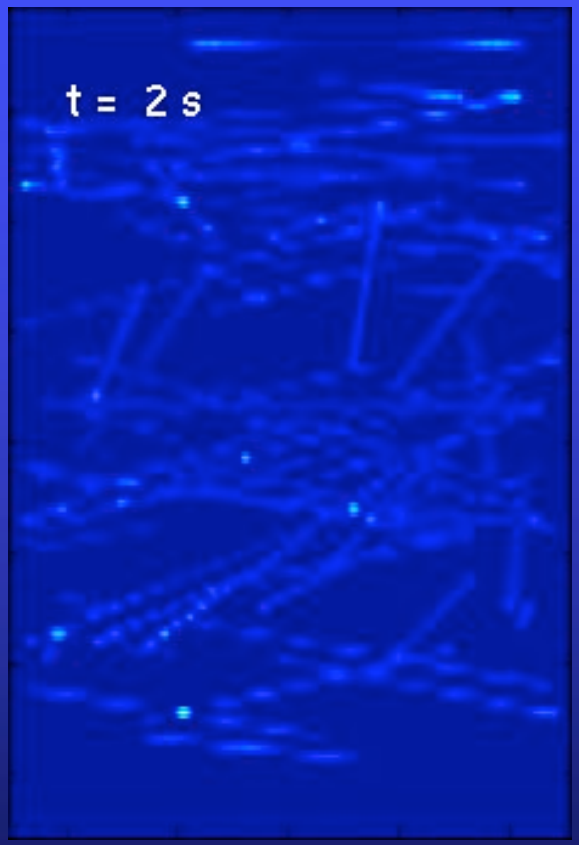
Motivations For Animal Monitoring

- Increase in the number of rodent models of disease.
  - Observe disease progression in individual animals non-invasively.
- Speed assessment of treatment interventions in animal models.
  - Study treatments in small numbers of animals longitudinally.
- Multiple tracer investigations in the same animal.

Dynamic Imaging



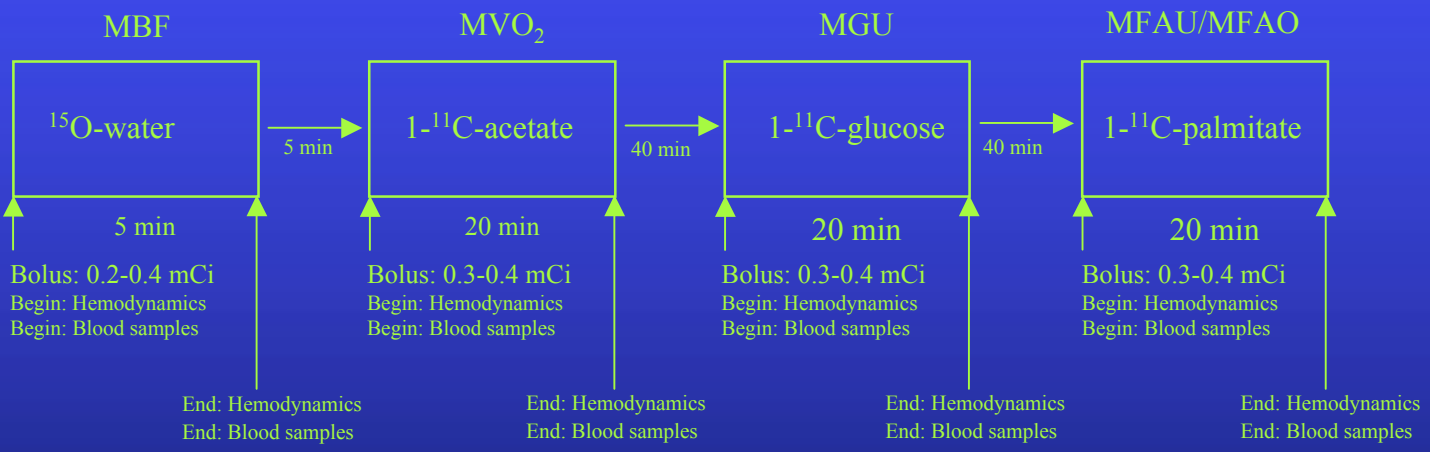
IV Injection



Interstitial Injection

Motivation from Cardiac Imaging

- GAP Studies in mouse and rat models of cardiac disease.



  - It is important to understand the physiologic stability of the animal over this time period.

Considerations

- Biological
  - Animal Model, Gender, Feeding Conditions, Anesthesia, Body Temperature, Injection Site.
- Animal Preparation
  - Microsurgical techniques, hemodynamics, animal monitoring.
- Physical
  - Radiation Dose (PET, SPECT, or CT).

Basic Physiological Data

	Mouse	Rat
Body Temperature	96.8°-100.4° F (36°-38° C)	96.6°-99.5° F (35.9°-37.5° C)
Heart Rate	325-780/min	250-450/min
Respiratory Rate	90-220/min	70-115/min
Blood Pressure (mmHg)	113-147/81-106	84-134/60

Harkness, Wagner; The Biology and Medicine of Rabbits and Rodents; 2nd Edition; 1983
Poole; The UFAW Handbook on the Care & Management of Laboratory Animals; 6th Edition; 1986

Biological Considerations

Animal Model, Gender,

Feeding Conditions,

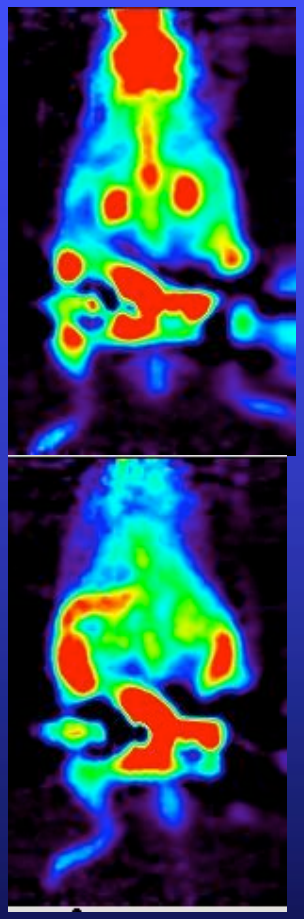
Anesthesia, Temperature,

Injection Site

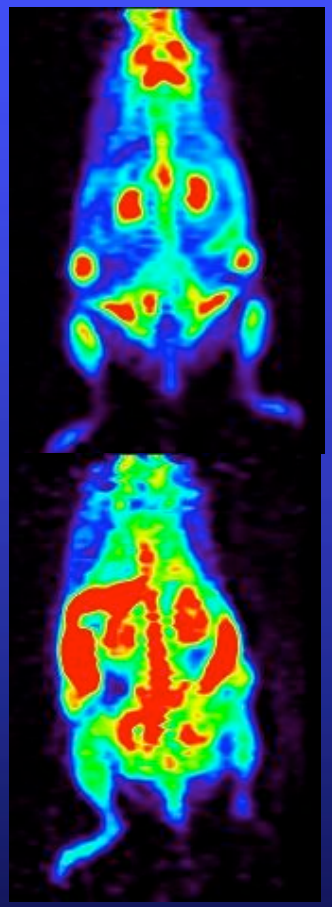
Min-O Mammary Cancer

FGD

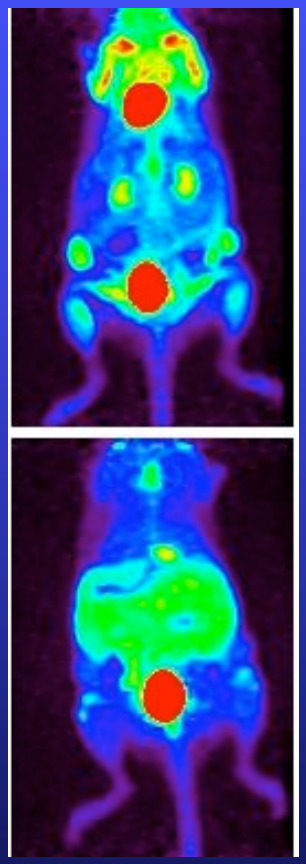
FLT



FBP

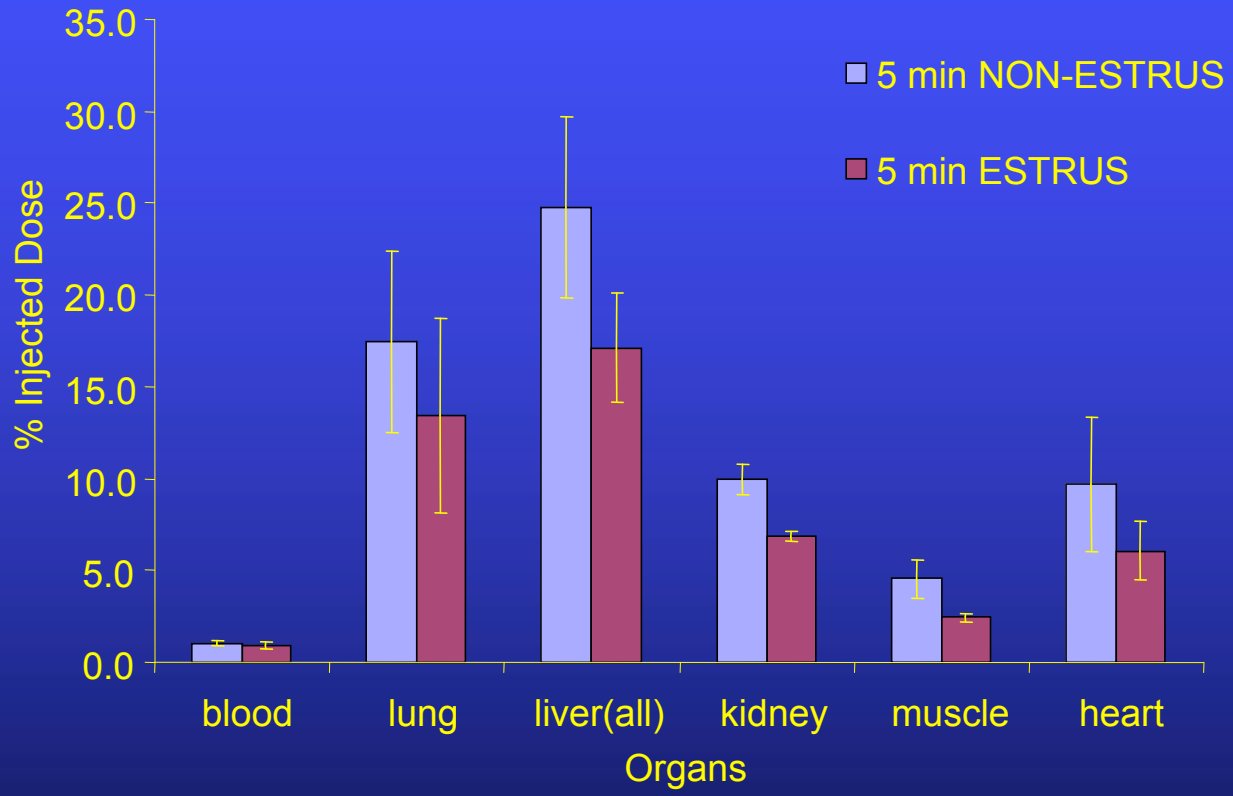


MAP



MIP

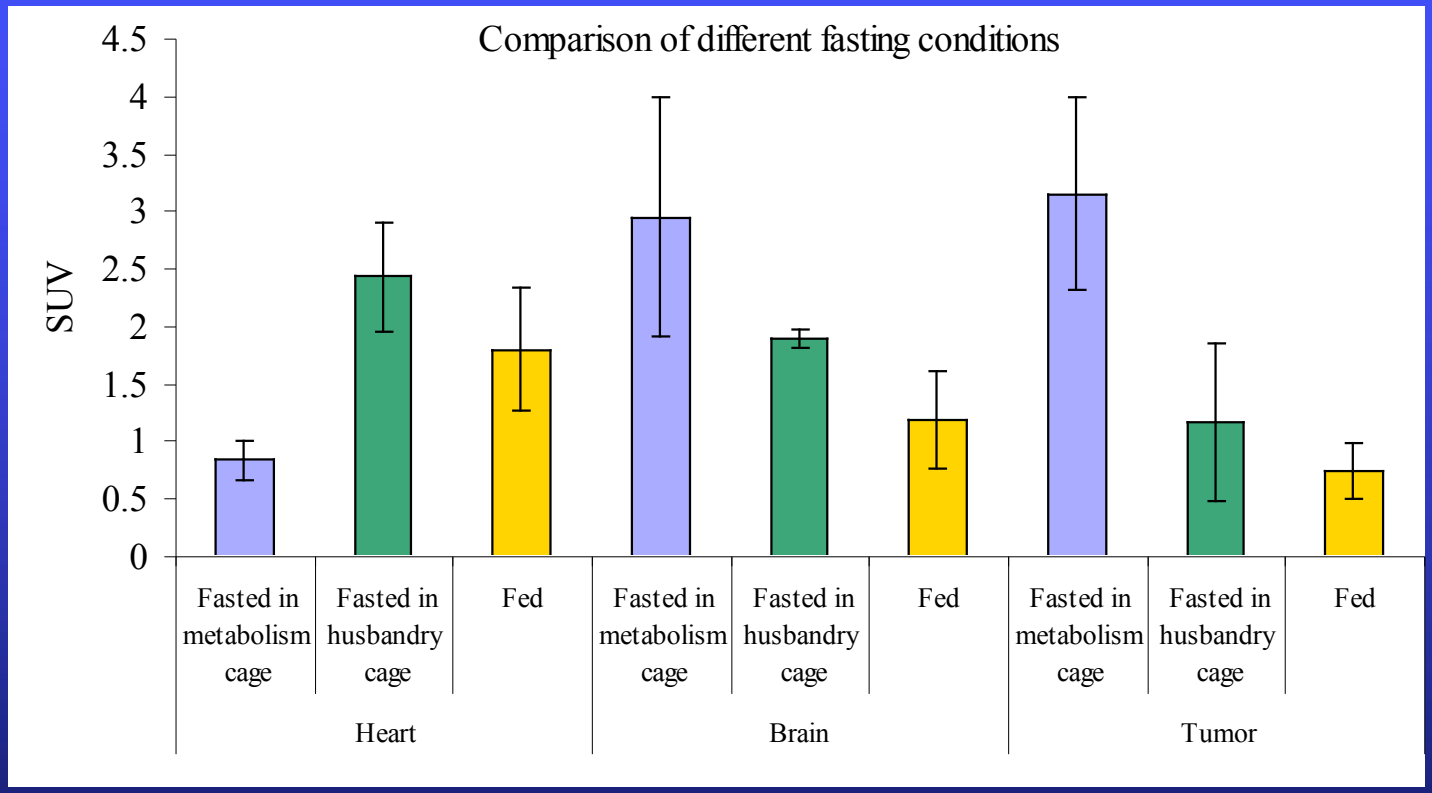
Estrus vs. Non-Estrus: ^{11}C-palmitate



Data courtesy of Washington University in St. Louis

Schaffer *et al.*, Upregulated Fatty Acid Transport Protein, Circ. Res. 96(22) (2004) 5-23.

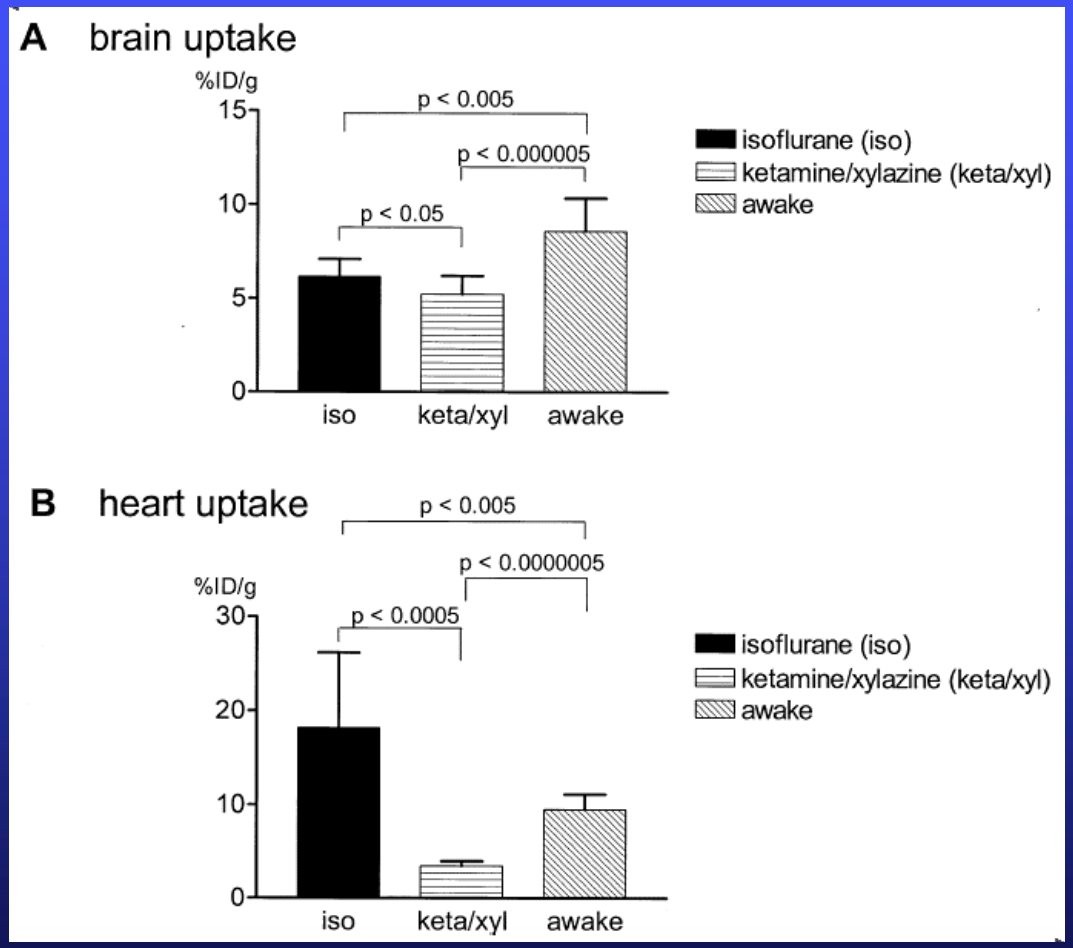
FDG in Cancer Models



Three Mice Fasted in Metabolism Cage, 3 Mice Fasted in Standard Husbandry Cage, 3 Mice Fed Scanned 35 Min Post Injection of ^{18}F-FDG

Data courtesy of Washington University in St. Louis

Anesthesia and [^{18}F]FDG Uptake



Toyama *et al.*, NMB 31(2) (2004) 251-256.

Impact of Animal Handling on the Results of ^{18}F-FDG PET Studies in Mice

Barbara J. Fueger[1], Johannes Czernin[1], Isabel Hildebrandt[1], Chris Tran[2], Benjamin S. Halpern[1], David Stout[1], Michael E. Phelps[1], and Wolfgang A. Weber[1]

[1]*Department of Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA, Los Angeles, California; and*
[2]*Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California*

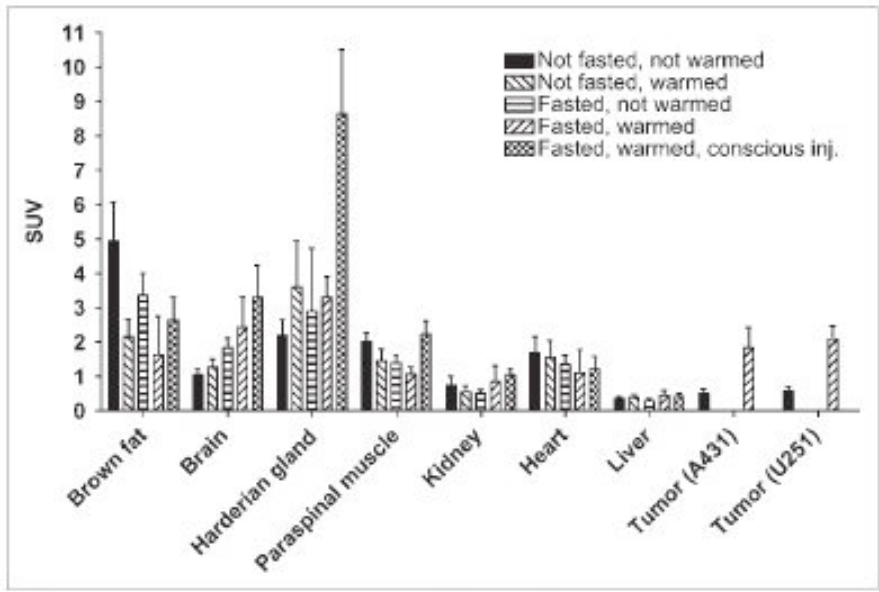


FIGURE 2. Biodistribution of ^{18}F-FDG in mice that were not anesthetized during uptake period for various studied conditions. Error bars show SD.

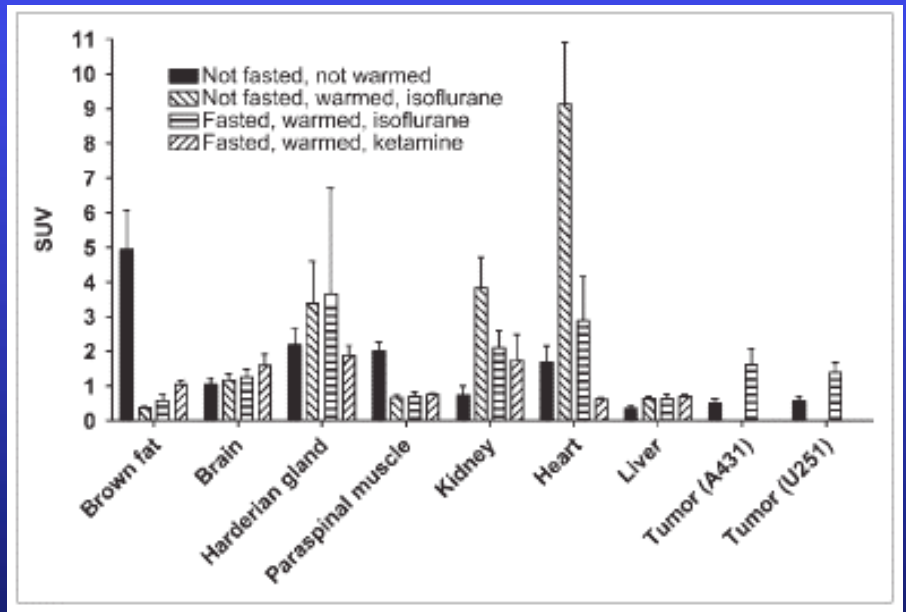


FIGURE 3. Biodistribution of ^{18}F-FDG in mice that were anesthetized during uptake period. Reference condition (not fasted, not warmed, no anesthesia during uptake period) is shown as a comparison. Error bars show SD.

Fueger *et al.*, JNM 47(6) (2006) 999-1006.

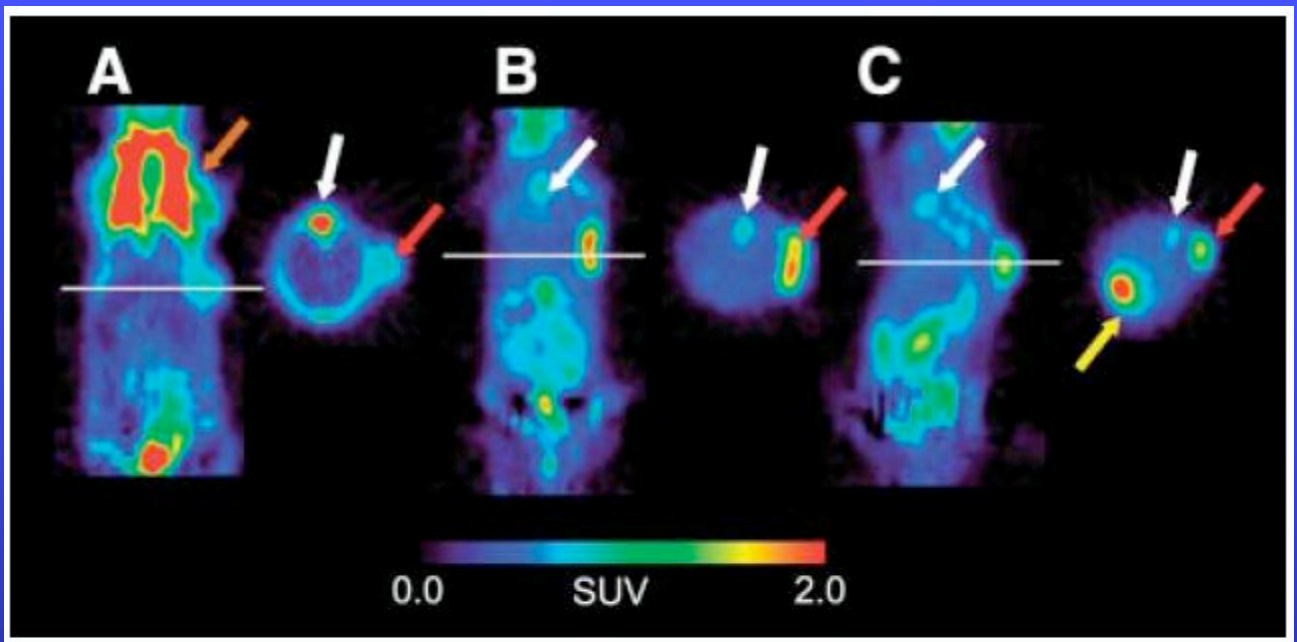


FIGURE 5. Tumor ^{18}F-FDG uptake under various conditions. Coronal and axial sections are shown. White lines in the coronal sections indicate position of axial sections. (A) Not fasted, not warmed, no anesthesia. (B) Fasted, warmed, no anesthesia. (C) Fasted, warmed, isoflurane anesthesia. Red arrow indicates tumor; brown arrow indicates brown fat; white arrow indicates paraspinal muscle; yellow arrow indicates myocardium.

Fueger *et al.,* JNM 47(6) (2006) 999-1006.

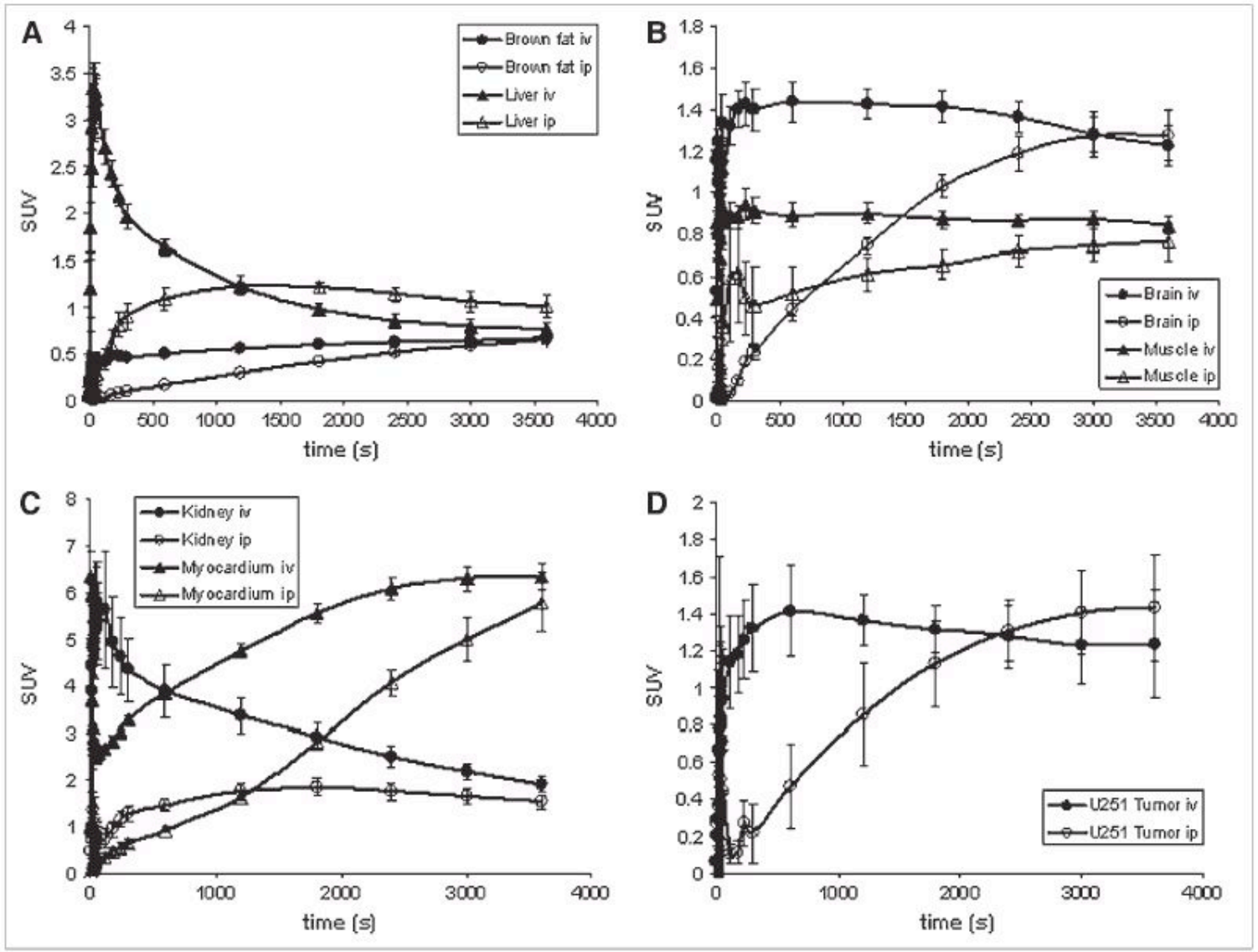


FIGURE 4. (A–D) ^{18}F-FDG uptake of various normal tissues and U251 xenografts after intravenous (iv) and intraperitoneal (ip) injection of ^{18}F-FDG (n = 6 per group). Error bars shown as SEs of the mean.

Fueger *et al.*, JNM 47(6) (2006) 999-1006.

Anesthesia: Supportive Care

- Maintain body temperature
  - Warm water recirculation pad
  - Heating foil built into animal bed
  - Deltaphase isothermal pad
  - Heat lamp (rheostat useful for control)
  - Warm air
  - Bubble wrap; plastic wrap
- Ocular lubricant
- Monitor respiratory rate
- Monitor, and keep animal warm, during recovery

Animal Preparation

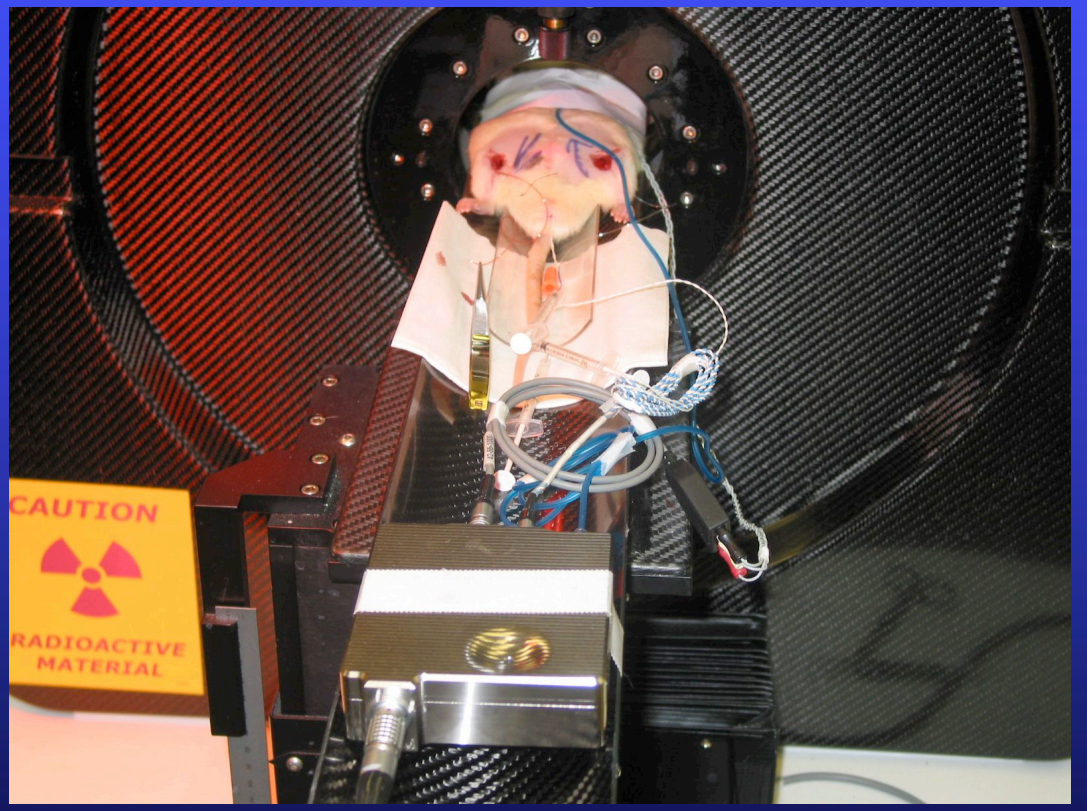
Microsurgical Techniques,
Hemodynamics, Animal Monitoring.

Physiological Monitoring

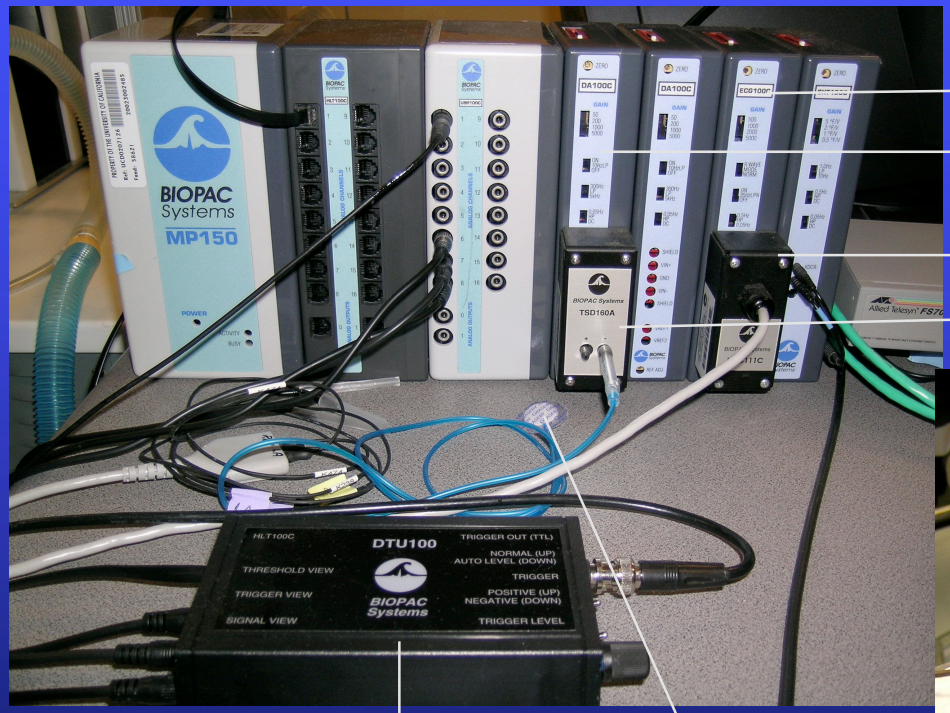
- ECG: Heart rate (gated)
- Respiratory rate (gated; Graseby Medical)
- Body temperature (rectal probe)
- Blood pressure (eg, Millar catheters)
- Blood glucose (eg, ACCU-CHEK Advantage)
- Pulse oximeter (eg, MouseOx;Starr Life Sci.)

Hemodynamic Monitoring

- Physiological Parameters
  - Spin Systems Biovet
    - Heart Rate
    - Respiratory Rate
    - Basal Body Temperature
  - Welch Allyn ProPaq 206
    - Blood Pressure
  - Saturated Oxygen Absorption
  - Hematocrit



ECG and Respiration Data Acquisition



ECG100C ECG Amplifier

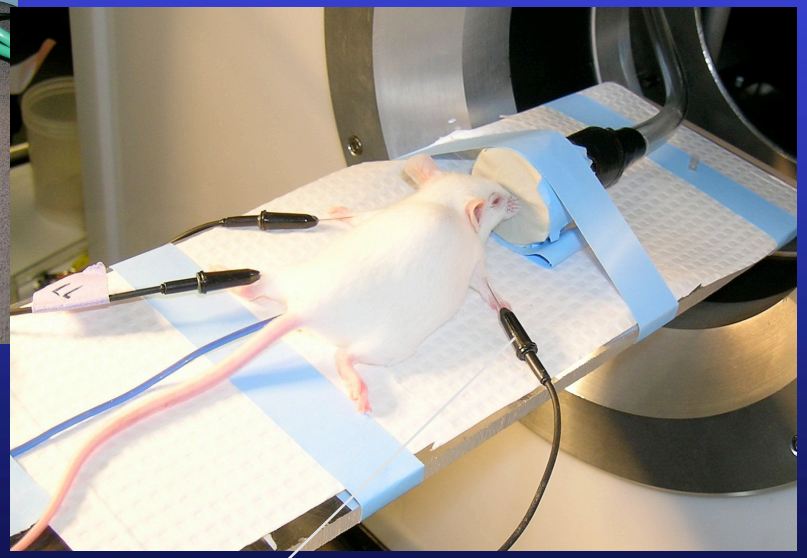
DA100C General-Purpose Amplifier for Respiration Signal

MEC111C ECG Lead Cable

TSD160A High Sensitivity Pressure Trans.

DTU100
Digital Trigger Unit

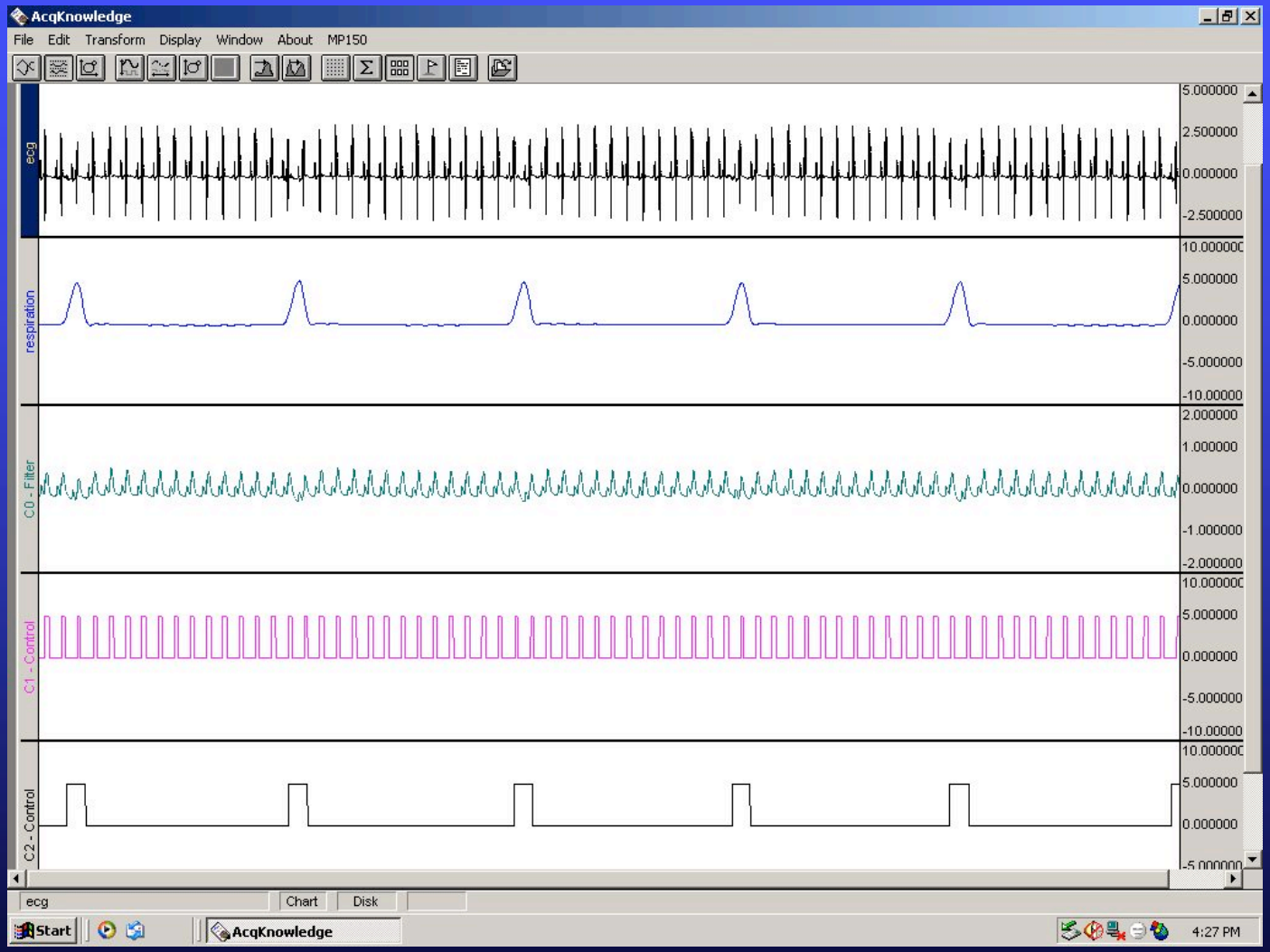
Respiration
Sensor (Graseby)



ECG Needle Electrode

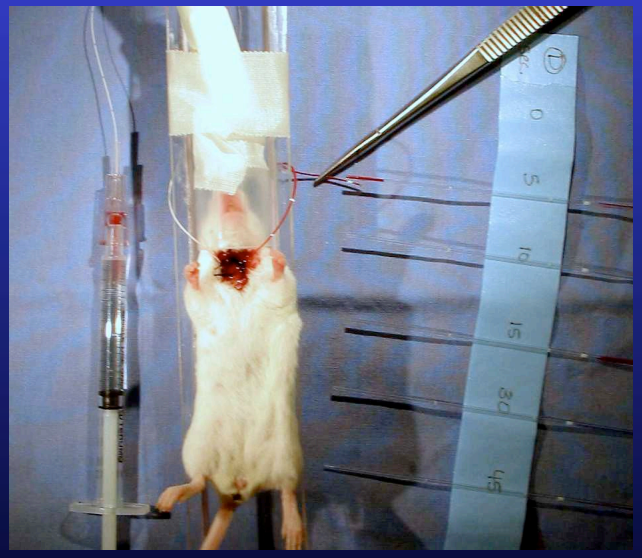
BIOPAC Systems, Inc.

ECG and Respiration for Gating



Micro-Surgical Techniques: Stable and Secure Vascular Access

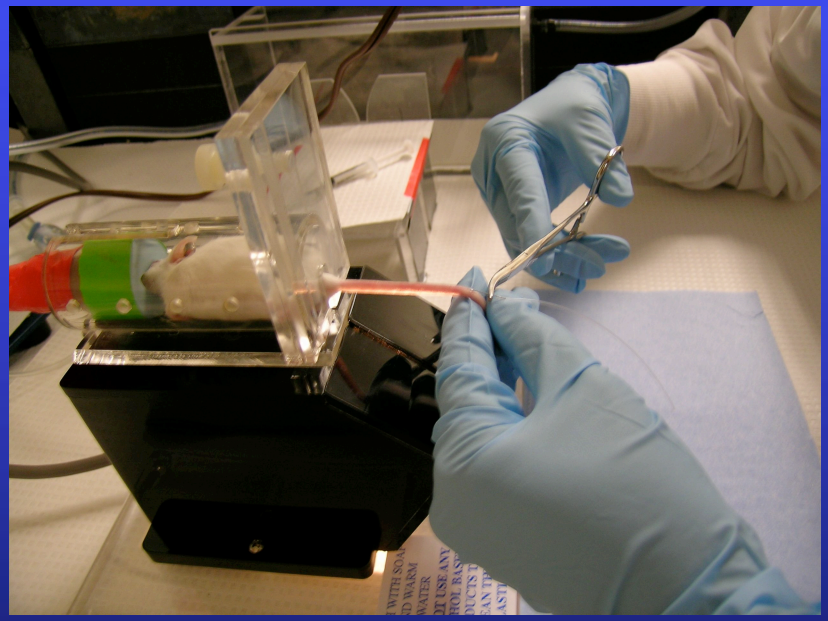
- Arterial/Venous Catheter Placement
  - Micro-Catheter (Harvard Apparatus, Inc.)
  - Micro-Renathane Tubing (Braintree Scientific, Inc.)

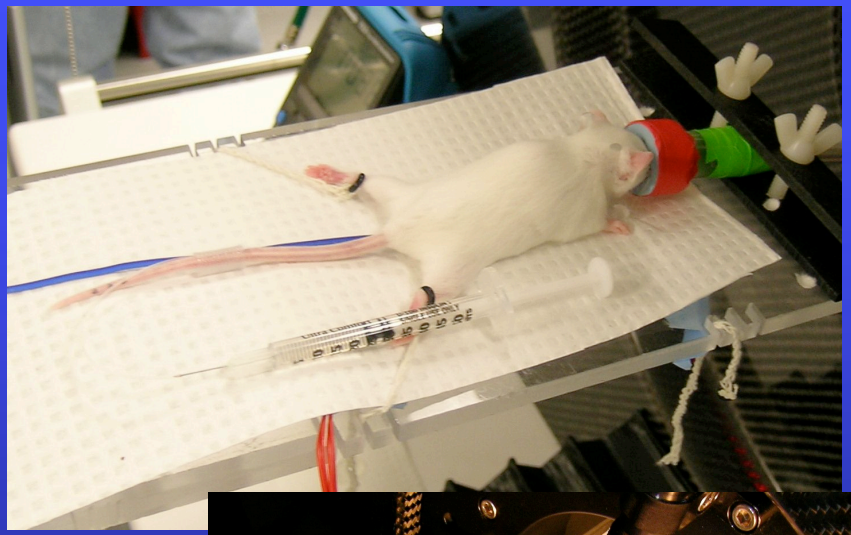


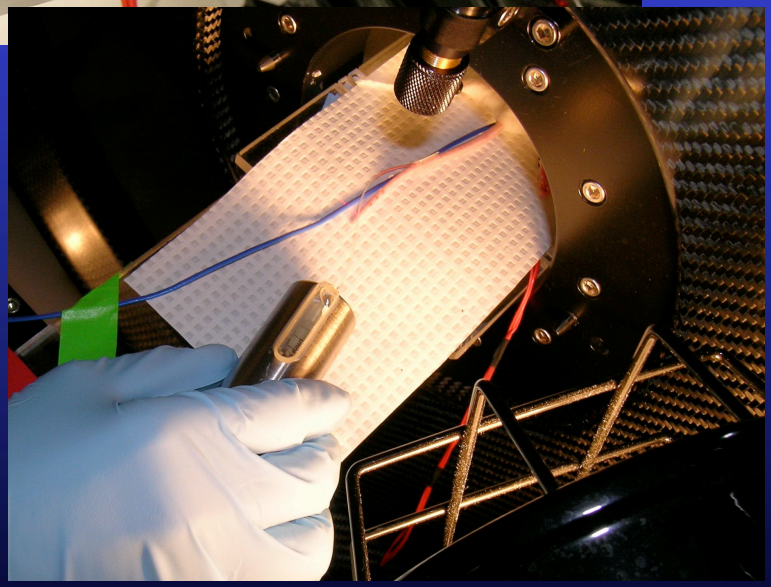
- Venous Injection
- 5-25μL micro-centrifuge tubes
- Carotid Artery Sampling, Descending Aorta Sampling, Venous Sampling

- Time Activity Curves (TAC)
- Blood Flow Estimation
- Substrate Levels
- Metabolite Analysis

Mouse Tail Vein Catheter







Needle almost parallel to vein

Recommended Injection Volumes (and Maximal Dose Volumes)

	Routes and Volumes (ml/kg)			
	IV[a]	IP	SC	IM
Mouse	5	20 (80)	10 (40)	0.05 (0.1)[b]
Example	Volume of administration (ml)			
25 g	0.125	0.5 (2)	0.25 (1)	0.05 (0.1)
Rat	5	10 (20)	5 (10)	0.1 (0.2)[b]
Example	Volume of administration (ml)			
250 g	1.25	2.5 (5)	1.25 (2.5)	0.1 (0.2)

[a] Bolus injection, over approximately 1 min

[b] Values in ml/site

Diehl et al; J Appl Toxicol, 21:15-23, 2001

Blood Collection Sites

	Anesthesia Required?	Comments
• Tail Vein	No	Repeatable
• Submandibular	No	Repeatable; large vols.
• Saphenous Vein	No	Slower; Repeatable
• Tail Tip	Yes	Multiple samples; allowed?
• Retro-orbital	N/Y	Rapid; potential compli-cations; skilled tech.
• Cardiac puncture	Yes	Terminal; open or closed chest; more blood
• Catheters	Yes	Slower; Multiple samples

Recommended Maximum Blood Withdrawal

	Circ. Blood Volume*	1% of Body Weight	10% of Blood Volume
Mouse			
20 g	1.6 ml	0.2 ml	0.16 ml
35 g	2.8 ml	0.35 ml	0.28 ml
Rat			
200 g	14 ml	2 ml	1.4 ml
350 g	24.5 ml	3.5 ml	2.5 ml

*Circulating blood volume: Mouse (76-**80** ml/kg)
Rat (54-**70** ml/kg)

Harkness, Wagner; The Biology and Medicine of Rabbits and Rodents; 2nd Edition; 1983

Validation of Techniques: Test – Retest Experiment

- Normal Sprague Dawley Rats
- Arterial Blood Sampling
- Day 1 and repeated after 6 days (Day 2)
  - 3 groups studied
    - FED fed normal rodent chow ad libitum
    - FAS fasted in metabolism cages for 12h
    - IL intravenous infusion of 20% Intralipid (0.001 mL/min)
  - Anesthetized for 6 hours each study day
  - Blood samples withdrawn (BSLN, 1hr, 2hr, 3hr, 4hr)
  - Recovered and repeated 6 days later

Stability of Hemodynamics

Average of each animal over the 4h study period

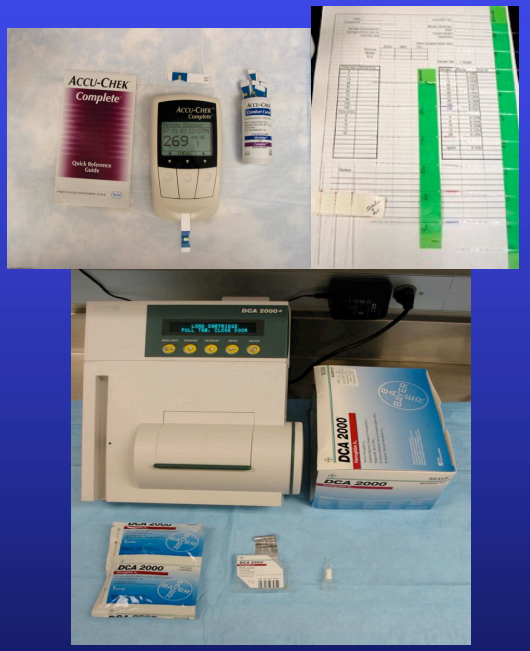
	FED		FAS		IL	
	D1	*D2*	*D1*	*D2*	*D1*	*D2*
HR	330 ± 40	303 ± 24	276 ± 17	289 ± 21	255 ± 10	279 ± 8
%SaO_2	0.94 ± 0.03	0.93 ± 0.01	0.93 ± 0.01	0.92 ± 0.04	0.94 ± 0.03	0.94 ± 0.02
Hct	42.5 ± 0.71	41.1 ± 0.57	42.6 ± 0.76	39.2 ± 1.79*	41.4 ± 2.06	41.2 ± 0.95

*$p < 0.05$ (D1 *vs* D2)

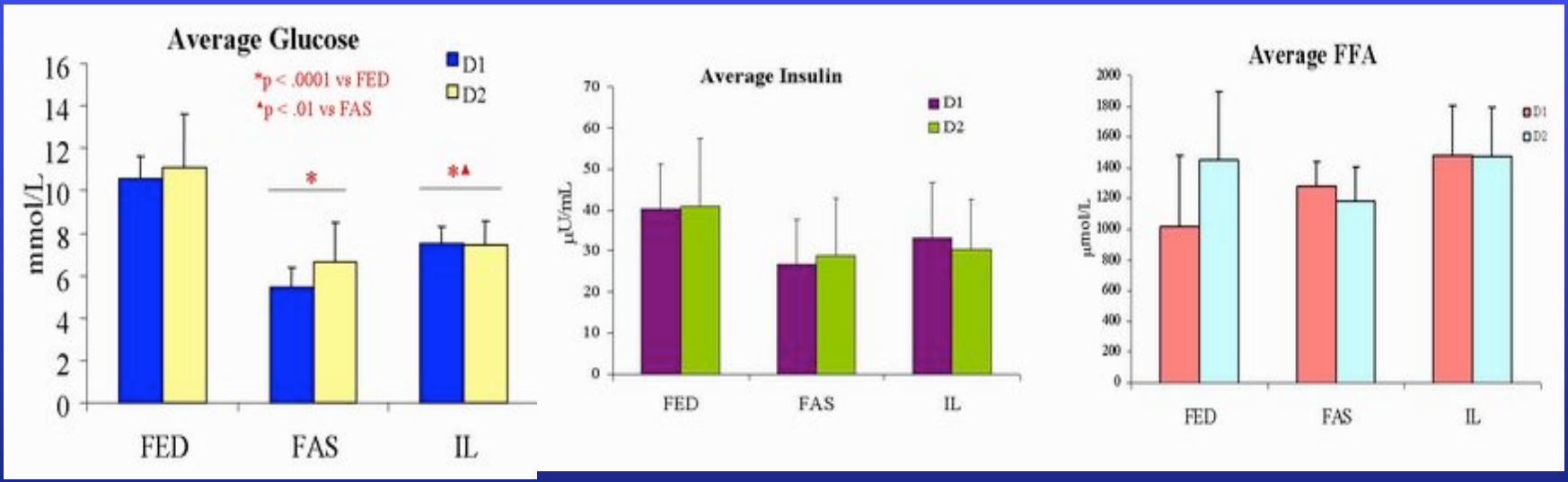
Sharp, TL, *et al.*, NMB 32(8) (2005) 875-884.

Circulating Plasma Substrate Analysis

- **Glucose (mg/dL -- µmol/L)**
  Accu-Check Plasma Glucometer, Roche Diagnostics, Indianapolis, IN, USA
- **Insulin (pcg/mL - ng/mL - µU/mL)**
  ELISA RAT INSULIN KIT, Crystal Chemicals, Chicago, IL, USA
- **Free Fatty Acids (µmol/L)**
  Nefa C Test Kit, Non-esterified Free Fatty Acids, Wako Chemicals USA, Inc., Richmond, VA, USA
- **HbA1c -- DCA2000+ Analyzer**
  Modern Laboratory Services, Inc., Bakersfield, CA, USA

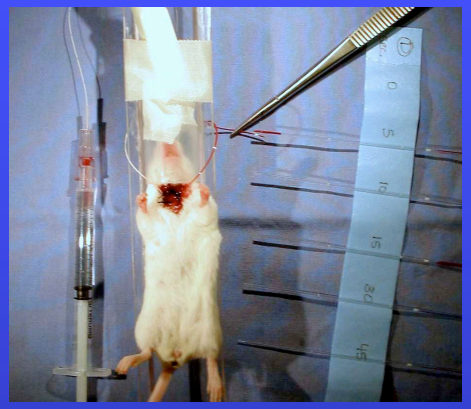


Variance of Plasma Substrate Levels



Sharp, TL, *et al.*, NMB 32(8) (2005) 875-884.

Blood Input Function Measurement



- Invasive
  - microBlood sampling techniques
  - Beta Probe inside or along side a major blood vessel with/without Arterial/Venous shunt
- Non-invasive
  - Factor Analysis of Dynamic Structures (FADS)
  - Direct extraction from novel image reconstruction algorithms and cardiac gating

Arterial Input Function Measurement Without Blood Sampling Using a β-Microprobe in Rats

Frédéric Pain, PhD[1]; Philippe Lanièce, PhD[1]; Roland Mastrippolito, PhD[1]; Philippe Gervais, PhD[2]; Philippe Hantraye, PhD[3,4]; and Laurent Besret, PhD[3]

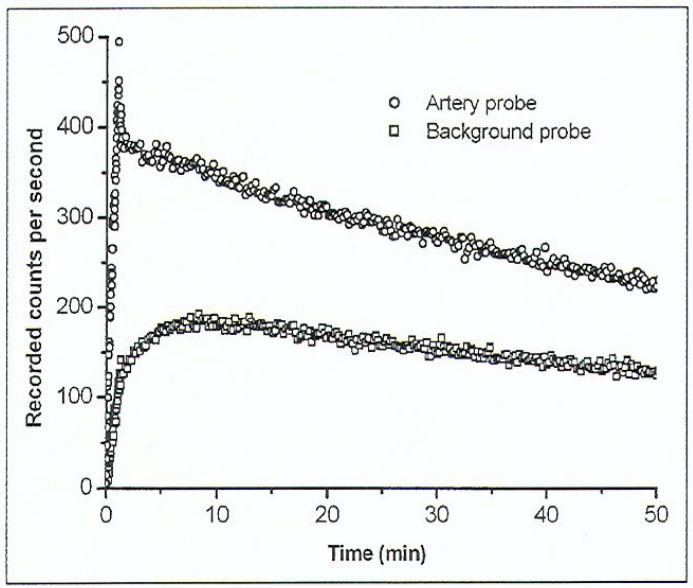


FIGURE 3. Raw time–activity curves recorded by the artery and background probes after bolus injection of ^{18}F-FDG.

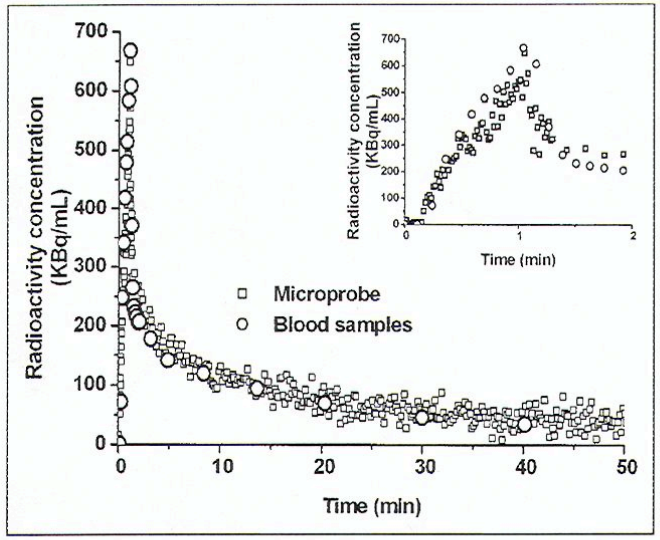
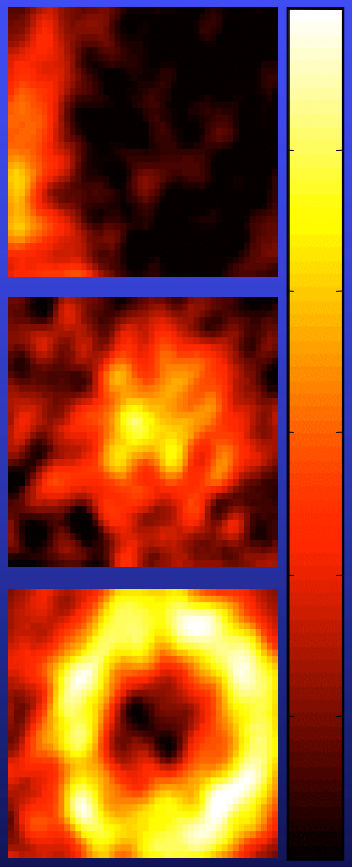


FIGURE 4. Arterial input functions determined with blood sampling or the β-microprobe. Graph shows 1 datum point every 10 s and then averaging of the data every 10 s. Inset zooms in on the first 2 min after bolus injection.

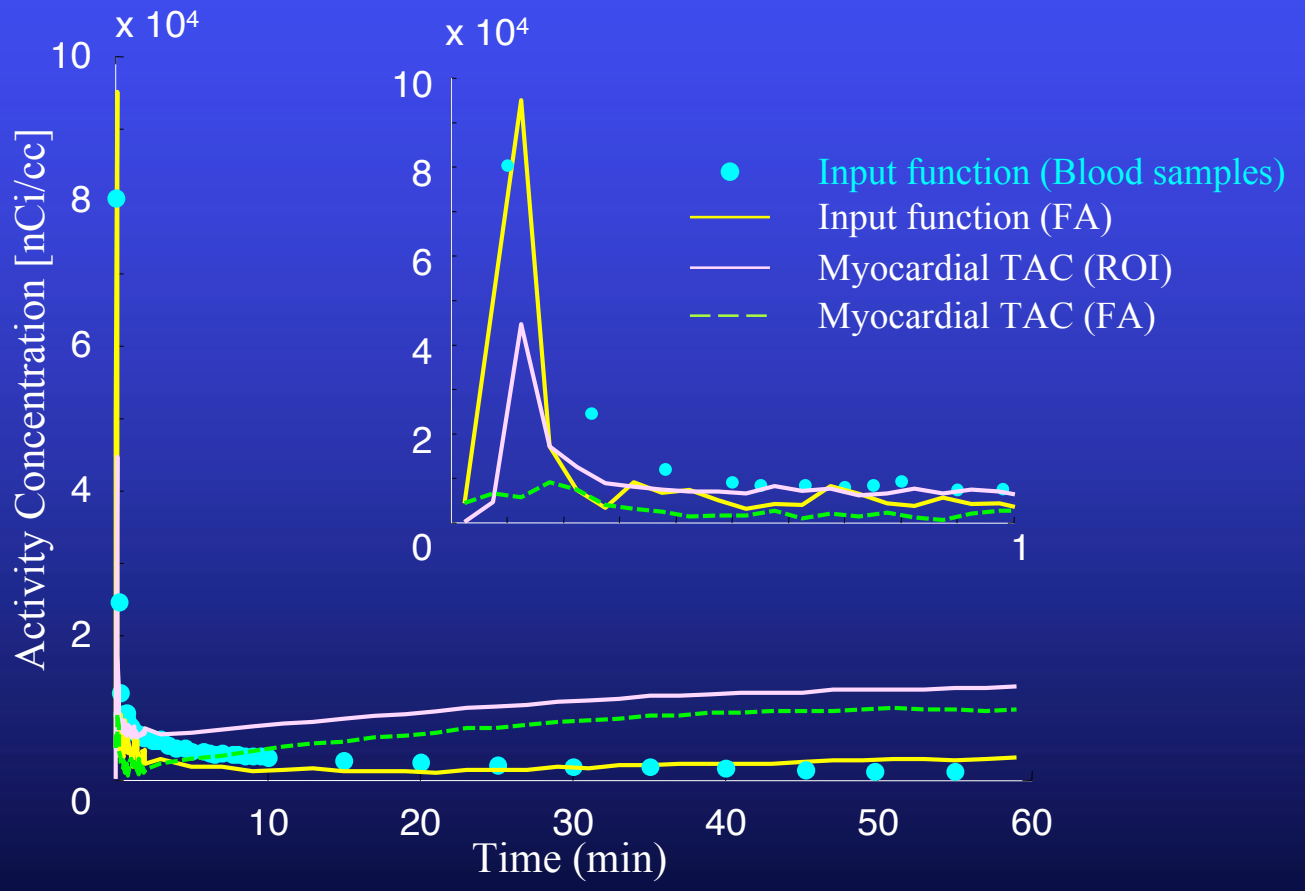
Pain *et al.,* J. Nucl. Med. (45) 2004 1577-1582.

Factor Analysis in Multivariate Analysis

• ^{18}F-FDG dynamic images on the microPET FOCUS. The % error of AUCs was 11.0 % for input function (FA) compared to that obtained with blood sampling.

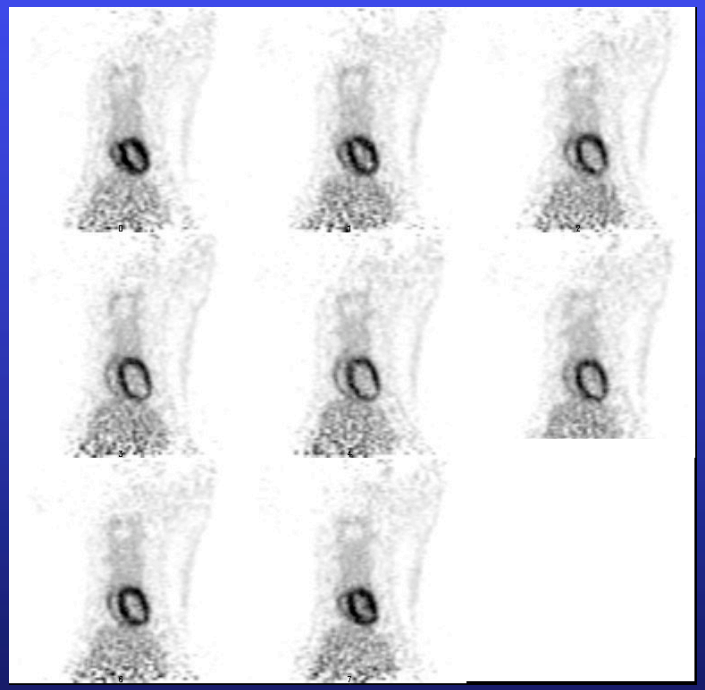


Factor Images of Rat

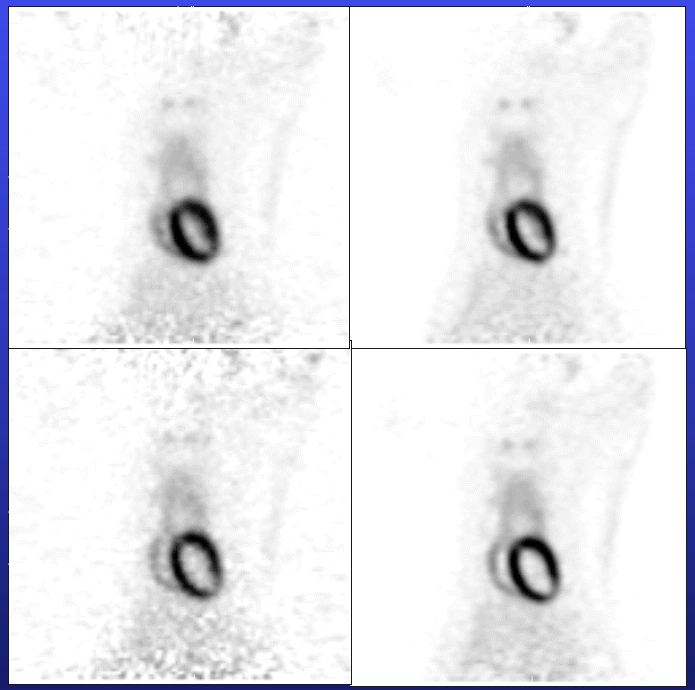


VOI Direct Measurement of Input Function

MAP Gated



FBP MAP

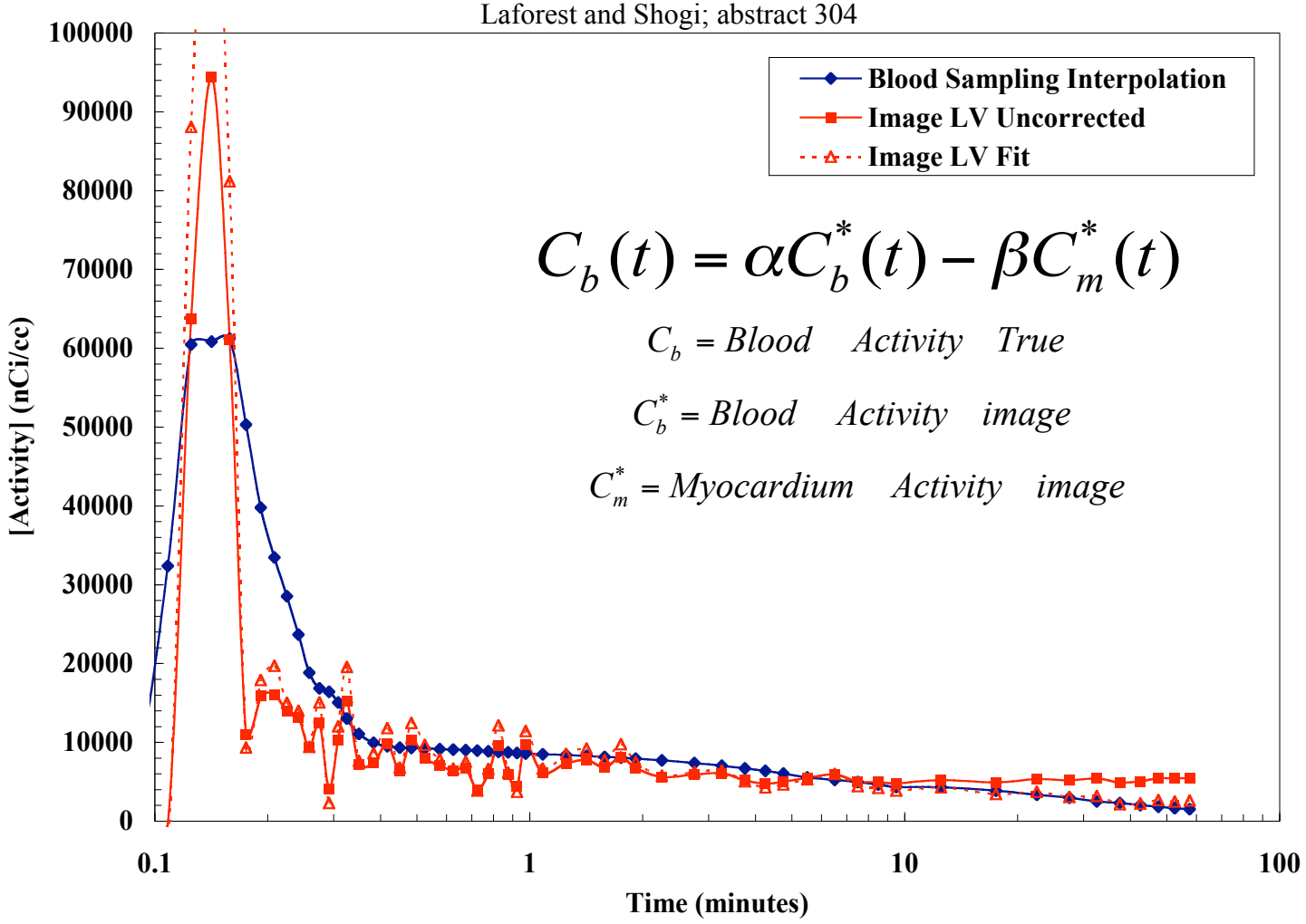


Non-Gated

Diastole

VOI Derived Input Function for the Rat

Laforest and Shogi; abstract 304



$$C_b(t) = \alpha C_b^*(t) - \beta C_m^*(t)$$

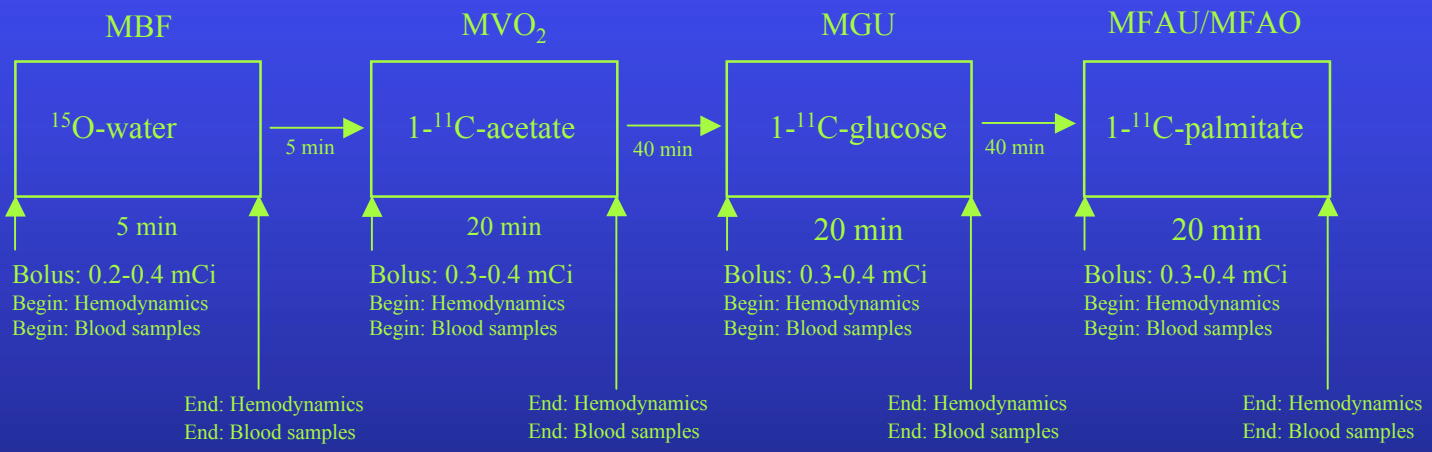
C_b = *Blood Activity True*

C_b^* = *Blood Activity image*

C_m^* = *Myocardium Activity image*

Motivation from Cardiac Imaging

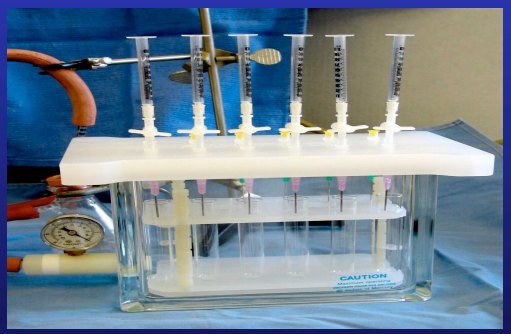
- GAP Studies in mouse and rat models of cardiac disease.



* It is important to understand the physiologic stability of the animal over this time period.

Radioactive Acidic Metabolites

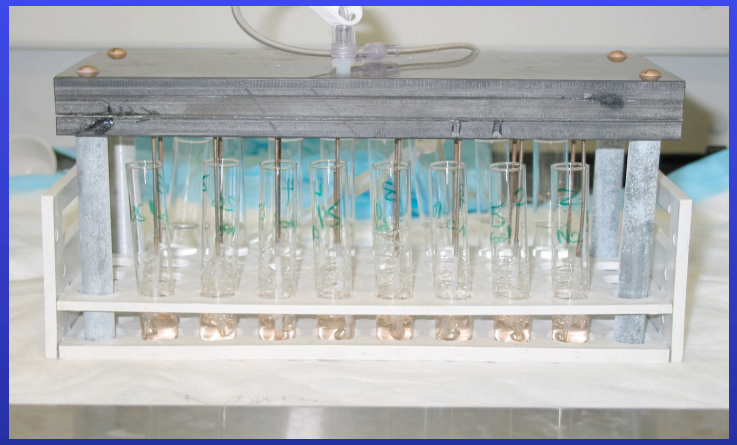
- Arterial plasma blood samples (2, 5, 10, 15, 20 min post tracer injection)
- "prototype" micro-columns
- AGI-8X-formate resin1
- De-Ionized H_2O
- Small amount of glass wool
- Gamma Counter --
- Column -vs- Diluent



Sharp, TL, *et al.*, NMB 32(8) (2005) 875-884.

Labeled $^{11}CO_2$

- Dedicated ductless Hood for analysis
- Arterial blood samples (2, 5, 10, 15, 20 min post tracer injection)
- Basic Solution -- Counted in well counter
- Acid Solution -- Bubbled with N_2O (10 min)
- Special Designed Manifold System Permits CO_2 to evenly distribute through all test tubes.
- Re-Count in well counter



Sharp, TL, *et al.*, NMB 32(8) (2005) 875-884.

Physical
Radiation Dose

Health of the animal in longitudinal studies, tumor progression (both inhibition and induction) in cancer models.

Dose Effects on Tumors

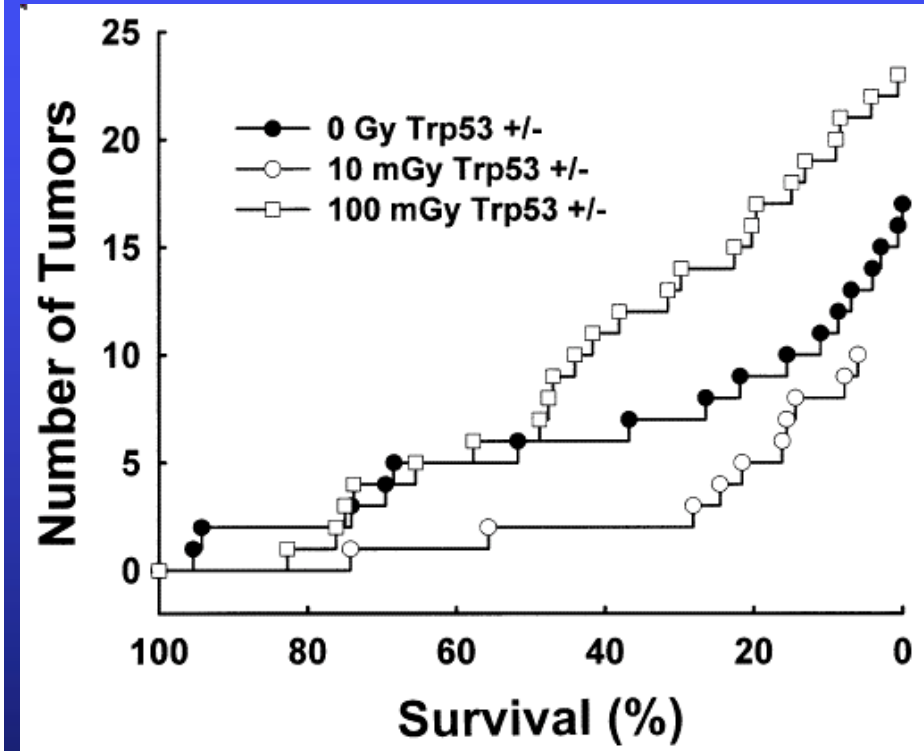


FIG. 3. The appearance of spinal osteosarcomas associated with paralysis in *Trp53* heterozygous mice exposed to 0, 10 or 100 mGy at low dose rate as a function of the overall survival of each group of mice.

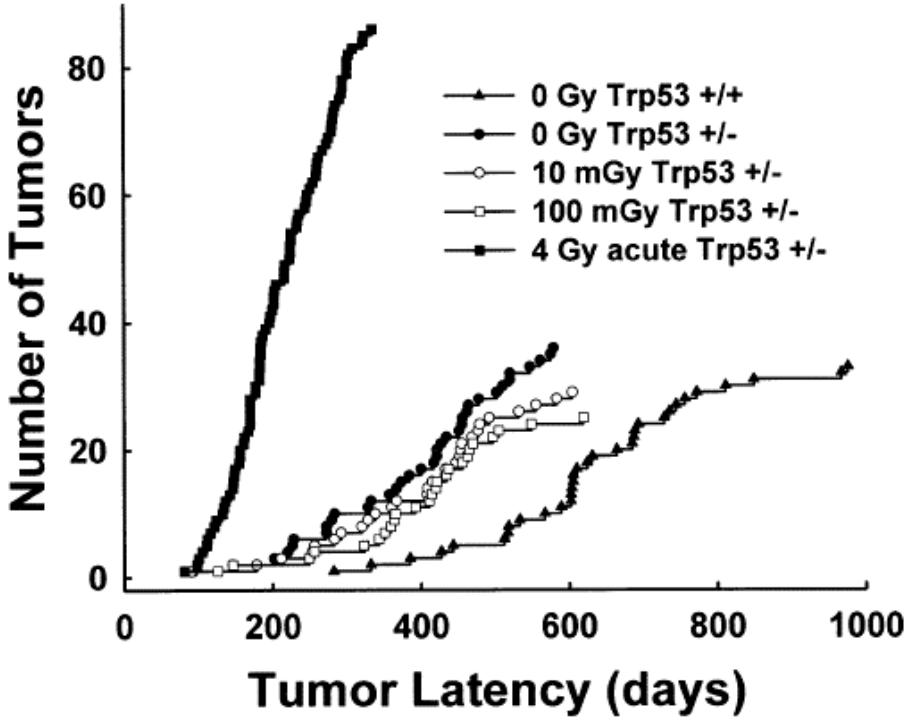
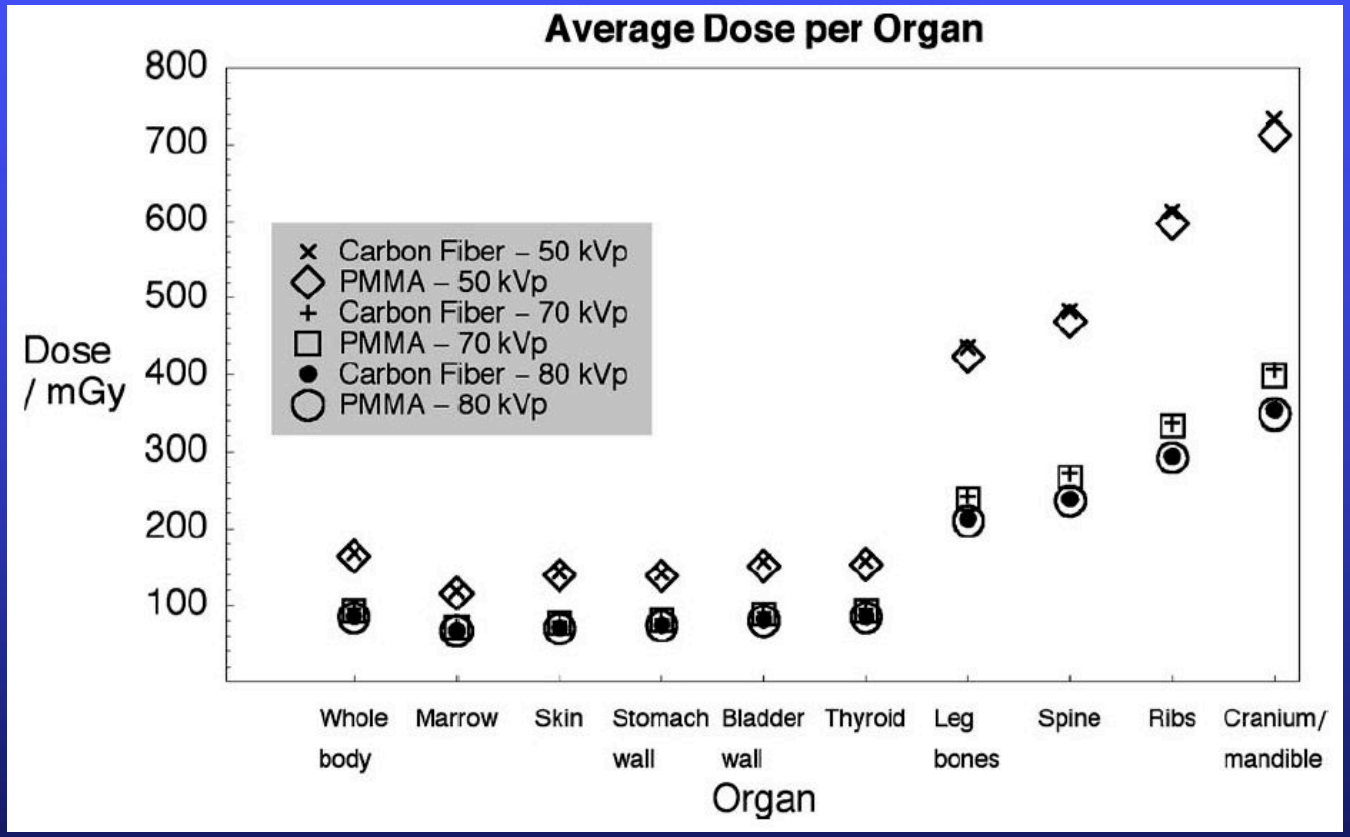


FIG. 4. Latency of lymphomas appearing in unexposed *Trp53* normal mice and in *Trp53* heterozygous mice exposed to various doses of radiation.

Mitchel *et al.*, Radiation Research, 159 (2003) 320-327.

CT Dose



Taschereau *et al.*, Med Phys 33(1) (2006) 216-224.

PET Dose

TABLE II. Calculated absorbed dose for a 7.4 MBq injection.

Structure name	FDG (mGy/7.4 MBq)	FLT (mGy/7.4 MBq)	Fluoride ion (mGy/7.4 MBq)
Bladder wall (low-res)	4018	3130	2531
Bladder wall (hi-res)	3915	3050	2466
Body	106	96	77
Brain	98	89	94
Cranium	30	30	457
Heart	425	96	61
Intestine wall	29	81	19
Kidneys	195	104	117
Lower limbs	26	22	473
Liver	42	95	58
Lungs	67	44	79
Pancreas	100	96	56
Ribs	38	35	206
Skin	19	18	21
Bone (spine)	36	32	560
Marrow (low-res)	74	67	306
Marrow (hi-res)	⋯	⋯	488
Spleen	96	88	52
Stomach wall	32	37	26
Testes	118	106	64
Thyroid	105	95	37
Tumor	223	488	⋯
Vas deferens	221	181	136

Taschereau and Chatziioannou: Simulation of Absorbed dose from 18-fluorine compounds. Medical Physics, Vol 34 No 3 (2007)

Conclusions

- Accurate and consistent data from imaging studies.
  - Must have a proper understanding of the biology of the animals being studied.
  - Animal preparation is important.
- Monitoring
  - Provide other important information on stability of the animal during the experiment.
  - Secondary information for correction of images.
- Physical understanding of the imaging technique
  - Radiation Doses and image registration.

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- UC Davis - CMGI
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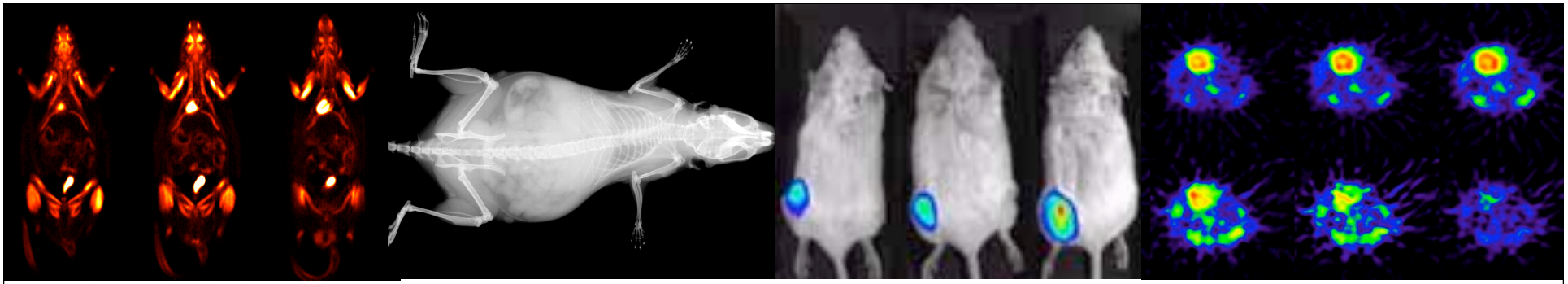

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Workshop Content

- Lectures on:
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  - Contrast agents and tracers
  - Animal restraint and support
  - Physiologic monitoring, animal restraint and anesthesia,
  - Image display and analysis
  - Imaging Center: Considerations in design and operation
- Practical demonstrations of
  - X-ray CT
  - PET
  - Optical imaging
  - MRI
  - Ultrasound