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Fluospheres for Cardiovascular Phenotyping Genetically Modified Mice

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Summary:

Assessment of systemic and regional hemodynamic phenotypes in genetically engineered mice by nonradioactive methods is yet an unsolved problem. We therefore investigated whether the reference sample method using fluorescent microspheres (FMs), already validated in rats, might be used for this purpose in C57BL/6 and in apolipoprotein E (ApoE)-deficient mice. FMs were injected into the left ventricle of instrumented anesthetized mice. In 10-week-old C57BL/6, cardiac output was 18-19 ml/min, and its regional distribution under basal conditions was ~1.5% (brain), 3.5% (heart), 9.1% (left kidney), 9.8% (right kidney), 1% (spleen), and 0.8% (stomach) (i.e., values similar to those previously reported with radioactive microspheres). Proper mixing of FMs was achieved as both kidneys had identical flows; distribution of two differently labeled FMs injected simultaneously was shown to be identical by an agreement study, and FM trapping in the capillary bed was demonstrated both histologically and by the recovery in the lungs of 90% of intravenously injected FMs. In addition, identical values for cardiac output and its distribution were obtained in different age-matched groups of C57BL/6. The FM technique also proved to be able to evidence angiotensin II and isoprenaline classic systemic and regional hemodynamic effects. Finally, applied to 30-week-old ApoE-deficient mice and age-matched C57BL/6, the FM technique showed no major hemodynamic difference between the two groups, except for coronary blood flow, which was significantly decreased in ApoE-deficient mice. In conclusion, we demonstrated for the first time the feasibility, accuracy, and reliability of the FM technique at characterizing the cardiovascular phenotype of genetically engineered mice.

Fluorescent microspheres (FMs), or fluospheres, have recently become a popular alternative to radioactive microspheres for measuring cardiac output and regional blood flows in experimental cardiovascular pharmacology. They do indeed avoid the use of radioactivity, which for environmental, security, and regulatory reasons has become problematic. Furthermore, the FM technique has been shown to provide reliable measurements of cardiac output and regional flows in large animals (1-4), and we recently validated its use in rats. (5).

Transgenic technology is now widely used to study genes involved in the regulation of cardiac function and of hemodynamics in physiologic as well as in pathologic conditions. For feasibility and cost reasons, transgenic mice are used more frequently than transgenic rats, but the potential of these genetically engineered mice remains limited in terms of cardiovascular phenotype characterization and of drugs' cardiovascular effects evaluation, especially because of the lack of adequate methods for investigating systemic and regional hemodynamics. As the FM technique might help in solving the problem, we investigated in this study the feasibility of its use in intact closed-chest mice for the determination of cardiac output and regional blood flows, under both basal and pharmacologically induced dynamic conditions. The study was conducted in C57BL/6 mice, the basic strain most frequently used for transgenic manipulation, and in one genetically modified strain, the apolipoprotein E (ApoE)-deficient mouse.

METHODS

Feasibility and validation of FM technique in C57BL/6 mice

Instrumentation of animals. Animal instrumentation and subsequent experiments were performed in accordance with the regulations published by the Ministère Fransçais de l'Agriculture.

Adult male C57BL/6 (Iffa Credo, L'Arbresle, France) mice, 22-27 g body weight, were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). The mice were placed on a thermally controlled heating pad and maintained at 37 ± 1C, and a tracheotomy was performed. The two carotid arteries were cannulated using a stereomicroscope (MZ75A Zoom 8; Leica Microsystems, Rueil-Malmaison, France). For both arteries, a PE_{10} jointed to PE_{50} was used. The right carotid cannula used for injection of microspheres was advanced into the apical region of the left ventricle under continuous monitoring of the blood pressure wave on a Video Screen to ascertain the anatomic position of the catheter (Statham P10EZ transducer, Gould amplifier 13-4615-10 model, ES 2000 V12; Gould Instruments, Cleveland, OH, U.S.A.). The left carotid artery was used for arterial pressure and heart rate (HR) measurements and for blood withdrawal.

Systemic and regional hemodynamics under basal conditions. Two sets of experiments were performed using an adaptation in mice of the FM technique previously described and validated in rats (5).

In the first series, one set of fluospheres was used. A solution of yellow-green fluospheres (80µl; i.e., 80,000 FMs, 15 ± 0.5 µm; Triton Technology, San Diego, CA, U.S.A.) was sonicated, vortexed, and injected into the left ventricle of 11 instrumented anesthetized mice with a Hamilton syringe (model 1750 LT, Harvard Apparatus, South Natick, MA, U.S.A.) followed by 100 µl saline. A reference blood sample was withdrawn from the left carotid artery, beginning 5 sec before the FM bolus injection and continuing for a further 55 sec (rate, 0.25 ml/min), in a preweighed heparinized Hamilton glass syringe (Model 33; Harvard Apparatus, South Natick, MA, U.S.A.). The animals were then killed, and brains, hearts, kidneys, spleens, stomachs, and livers were removed, blotted, weighed, and then processed with the reference blood sample, for fluorescence quantification after digestion and filtration, as previously described by Gervais et al. (5).

Fluorescence was determined with a Perkin-Elmer LS50B luminescence spectrophotometer (Perkin-Elmer, Beaconsfield, U.K.) equipped with a 96-well microplate reader. The fluorescence intensity was measured at the optimal excitation-emission wavelengths of the yellow-green dye (490 and 510 nm).

In a second series of experiments, we performed an agreement study between two readings of independent sets of systemic and regional hemodynamic values obtained at the same moment in the same animals but with two different labels. This study was conducted to evaluate the stability of the reading procedure and the similarity of the FM distribution. In brief, 150µl of a mixture containing 100,000 blue and 50,000 yellow-green FMs (both 15 ± 0.5 µm) were sonicated, vortexed, and injected as previously described in 11 other instrumented anesthetized mice, followed by 100 µl saline. Reference blood sampling and organs (brain, heart, kidneys, spleen, stomach, and liver) processing were conducted as mentioned earlier. Fluorescence measurement was performed at 360 and 415 nm (blue) and at 490 and 510 nm (yellow-green) wavelengths.

Extraction efficacy of 15-µm fluospheres. Extraction efficacy of 15-µm fluospheres was studied in nine male C57BL/6 anesthetized mice, 22-26 g body weight. A solution (80 µl) of yellow-green fluospheres (i.e., 80,000 FMs) was injected as a bolus into the superior vena cava. The animals were then killed, and the lungs and kidneys were removed and weighed. Tissue samples were processed, and fluorescence was assessed as previously described so that the total number of spheres trapped in each organ could be calculated.

In addition, to determine whether fluospheres were trapped in the capillaries, 80 µl of a solution of yellow-green fluospheres (i.e., 80,000 FMs) was injected in the left ventricle of five additional C57BL/6 mice. After killing, the heart and kidneys were removed, and cardiac output and right renal blood flow were determined. Afterward, hearts and left kidneys were frozen and cut into 5-m thick sections. Sections were stained with hematoxylin and eosin and examined under a photomicroscope (model BH-2; Olympus, Rungis, France). This tissue preparation allows the histologic localization of microspheres that under conventional conditions would have been dissolved by organic solvents such as toluene used for dehydration.

Systemic and regional hemodynamics in C57BL/6 under dynamic conditions

Instrumentation of anesthetized male C57BL/6 mice (23-30 g body weight) was performed as previously described, but, in addition, a third catheter was inserted into the jugular vein for saline or drugs infusion (Harvard 33 double syringe pump; infusion rate, 0.03 ml/min for 3-5 min to reach a steady state). Eighty microliters of a solution of yellow-green fluospheres (i.e., 80,000 FMs) was then injected into the left ventricle. Blood pressure and HR were continuously recorded, except during the FM injection. Cardiac output and renal and myocardial blood flows were determined after saline (controls, n = 17) or angiotensin II (1 ng/min · g; n = 9) or isoprenaline [at three different doses: 0.01 (n = 9), 0.1 (n = 9), and 1 ng/min · g (n = 17)].

Systemic and regional hemodynamics in ApoE-deficient mice

These experiments were performed in 30-week-old male homozygous ApoE-deficient mice fed with a normal chow diet, and in age-matched C57BL/6 controls (all from Iffa Credo, L'Arbresle, France).

The animals (body weight, 30-40 g; n = 9 in each group) were anesthetized and instrumented as previously described. After a 15-min stabilization period, 80 µl of a solution of yellow-green fluospheres (i.e., 80,000 FMs) was injected into the left ventricle. Cardiac output and regional blood flows in the brain, heart, and kidneys were determined as previously described.

Calculations

Mean arterial pressure (MAP) was calculated as (SAP + 2DAP)/3; cardiac output (CO) was calculated as: CO = QfT/Rf, where, for each dye, T is the total injected fluorescence, Rf is the fluorescence of the reference blood sample and Qf is the reference flow calculated as Qf (ml/min) = Reference blood sample weight (g) x 60/Blood density (1.06) x Sampling time (60 sec). Cardiac index (CI, ml/min g) was calculated as CO/animal body weight (g); regional organ blood flows (Qo, ml/min g) were calculated for each organ as Qo = Organ fluorescence x Qf/Organ weight (g) x Rf, and total peripheral resistance (TPR: mm Hg min g/ml) and regional vascular resistances (mm Hg min g/ml) were calculated by dividing MAP by CI or regional blood flows.

Statistical analysis

Values of all hemodynamic parameters are given as mean ±SEM.

In the agreement study, the flow values obtained with the two different labels were compared according to Bland and Altman (6). For each flow value, the correlation between each set of paired values obtained with the two labels was investigated (equation of linear relation, correlation coefficient, *r* and p values).

Differences within pairs (D_i) were tested, and error's probability distribution was estimated by calculating a mean interlabel error ($\bar{D_i}$). When error did not depend on the measured absolute flow value, we estimated the distribution of this error as normal and calculated its 95% confidence interval as $1.96 \pm \sqrt{\Sigma Di^2/n}$. When error depended on the measured absolute flow value (proportional error), we log-transformed the data and applied the same procedure to obtain, after back-transformation, the confidence interval of the error as a percentage, and finally as an absolute range of value for each organ flow.

Comparisons of the hemodynamic data obtained in the different experimental groups were performed with a Student's *t* test or, when more than two sets were compared, by analysis of variance followed by a Student's *t* test using the Bonferroni correction for multiple groups comparisons.

	Absolute values	% of cardiac output
Cardiac output (ml/min)	18.2 ± 1.0	—
Cardiac index (ml/min per g of BW)	0.71 ± 0.04	—
Total peripheral resistance (mm Hg · min · g BW/ml)	149 ± 8	—
Brain		
Flow (ml/min · g)	0.6 ± 0.1	1.4 ± 0.1
Resistance (mm Hg · min · g/ml)	197 ± 20	—
Heart		
Flow (ml/min · g)	5.7 ± 0.3	3.9 ± 0.2
Resistance (mm Hg · min · g/ml)	19 ± 1	—
Left kidney		
Flow (ml/min · g)	12.7 ± 1.1	10.5 ± 0.8
Resistance (mm Hg · min · g/ml)	9 ± 1	—
Right kidney		
Flow (ml/min · g)	12.8 ± 1.0	10.9 ± 0.7
Resistance (mm Hg · min · g/ml)	9 ± 1	—

Values expressed as mean ± SEM.
BW, body weight.

TABLE 1. Cardiac output and its regional distribution in brain, heart, and kidneys of anesthetized C57BL/6 mice

RESULTS

Feasibility and validation studies in C57BL/6 mice

Systemic and regional hemodynamics under basal conditions. In the first series of experiments (yellow-green fluospheres), MAP and HR values were 104 ± 4 mm Hg and 498 ± 19 beats/min, respectively, before FM injection, and 90 6 mm Hg and 518 24 beats/min, respectively, at the end of the reference blood sampling. Cardiac output was 18.2 ± 1.0 ml/min, and cardiac index was 0.71 ± 0.04 ml/min per g of body weight (range, 23-28 g). **Table 1** shows CO regional distribution, indicating that heart, left kidney, right kidney, and brain received 3.9, 10.5, 10.9, and 1.4%, respectively, of this CO. Mean flows of the left and the right kidneys (12.7 ± 1.1 and 12.8 ± 1.0 ml/min g, respectively), did not differ from each other.

In the second series of experiments (blue and yellow-green fluospheres), MAP and HR values were 101 ± 4 mm Hg and 361 ± 25 beats/min, respectively, before FM injection, and 91 ± 4 mm Hg and 390 ± 27 beats/min, respectively, at the end of the reference blood sampling. **Table 2** indicates the mean values of CO and of its distribution to the different organs obtained with the two fluorescent labels and the respective confidence intervals calculated for these values with a 95% probability. CO and regional blood flow values obtained were, whichever the fluorescent label considered, similar to those measured in the first experiment (compare **Tables 1 and 2)**. Again, the kidneys received the largest fraction of CO, followed by the heart and brain. The mean flows to the left kidney evaluated with the blue and the yellow-green labels were 10.4 ± 0.7 and 10.2 ± 0.7 ml/min · g, respectively, and did not differ from the values obtained in the same conditions in the right kidney (i.e., 10.8 ± 0.8 and 10.8 ± 0.7 ml/min · g).

Correlation study (**Fig. 1A**) established a linear relation between the two series of measurements of CO with the two different fluorescent labels ($r = 0.985$; $p < 0.001$), the corresponding equation (slope, 1.10 ± 0.02; intercept, -1.4 ± 0.4) being not statistically different from the identity line. The difference between the two evaluations does not depend on the CO estimated value (**Fig. 1B**), and the mean error ($\overline{D_i} = 0.503 \pm 0.406$) does not significantly differ from 0 (Fig. 1B). The 95% confidence interval of CO is ±2.7 ml/min (**Table 2**), indicating that the difference between two determinations of CO is expected to be <±2.7 ml/min, with a probability of 95%. A correlation study also has been performed on all organs' pooled regional flow data (**Fig. 2A**). It shows that there is also a linear relation between the two series of measurements with the two different fluorescent labels ($r = 0.996$; $p < 0.001$), the corresponding equation (slope, 0.99 ± 0.01; intercept, 0.03 ± 0.01) being not statistically different from the identity line. The difference between the two evaluations increases with the flows' estimated values and the mean error ($\overline{D_i} = 0.037 \pm 0.047$) does not significantly differ from 0 (**Fig. 2B**). The 95% confidence interval for pooled regional flow values was ±17%, and Table 2 indicates in absolute values the 95% confidence intervals for each organ flow.

	Absolute values	% of Cardiac output
Cardiac output (ml/min)		
Blue	18.8 ± 1.8	—
Yellow–green	19.3 ± 2.1	—
95% CI	± 2.7	—
Brain (ml/min · g)		
Blue	0.7 ± 0.1	1.7 ± 0.1
Yellow–green	0.7 ± 0.1	1.6 ± 0.1
95% CI	[0.59–0.83]	—
Heart (ml/min · g)		
Blue	4.8 ± 0.5	3.0 ± 0.2
Yellow–green	4.8 ± 0.5	3.0 ± 0.2
95% CI	[4.06–5.68]	—
Left kidney (ml/min · g)		
Blue	10.4 ± 0.7	7.9 ± 0.6
Yellow–green	10.2 ± 0.7	7.7 ± 0.6
95% CI	[8.71–12.19]	—
Right kidney (ml/min · g)		
Blue	10.8 ± 0.8	8.7 ± 0.6
Yellow–green	10.8 ± 0.7	8.6 ± 0.6
95% CI	[9.13–12.78]	—
Spleen (ml/min · g)		
Blue	2.8 ± 0.3	1.1 ± 0.1
Yellow–green	2.7 ± 0.3	1.0 ± 0.1
95% CI	[2.37–3.31]	—
Stomach (ml/min · g)		
Blue	1.1 ± 0.2	0.8 ± 0.1
Yellow–green	1.1 ± 0.2	0.8 ± 0.1
95% CI	[0.93–1.30]	—
Liver (arterial) (ml/min · g)		
Blue	0.08 ± 0.02	0.5 ± 0.1
Yellow–green	0.08 ± 0.02	0.5 ± 0.1
95% CI	[0.07–0.09]	—

Values expressed as mean ± SEM. Agreement between data obtained by using two differently labeled fluospheres (blue or yellow–green) injected simultaneously and calculated 95% confidence intervals (95% CI) (see Methods).

TABLE 2. Cardiac output and its regional distribution in brain, heart, kidneys, spleen, stomach, and liver in anesthetized C57BL/6 mice

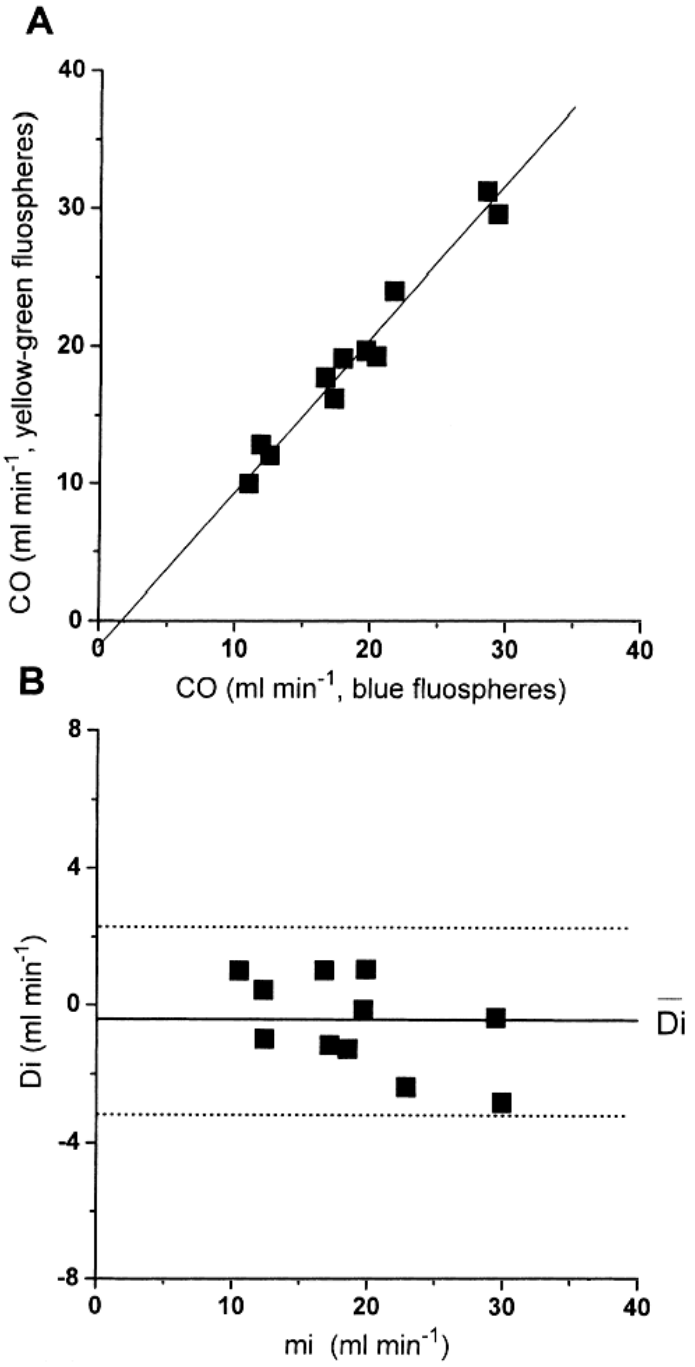

FIG. 1. **A:** Correlation of cardiac output (CO) values measured simultaneously with yellow-green and blue fluospheres. **B:** Plot of the difference (D_i) between each pair of cardiac output measurements against the mean of the pair (m_i). The solid line indicates the mean difference in flows, and dotted lines indicate the upper and lower limits of the 95% confidence interval calculated for one pair of measurements.

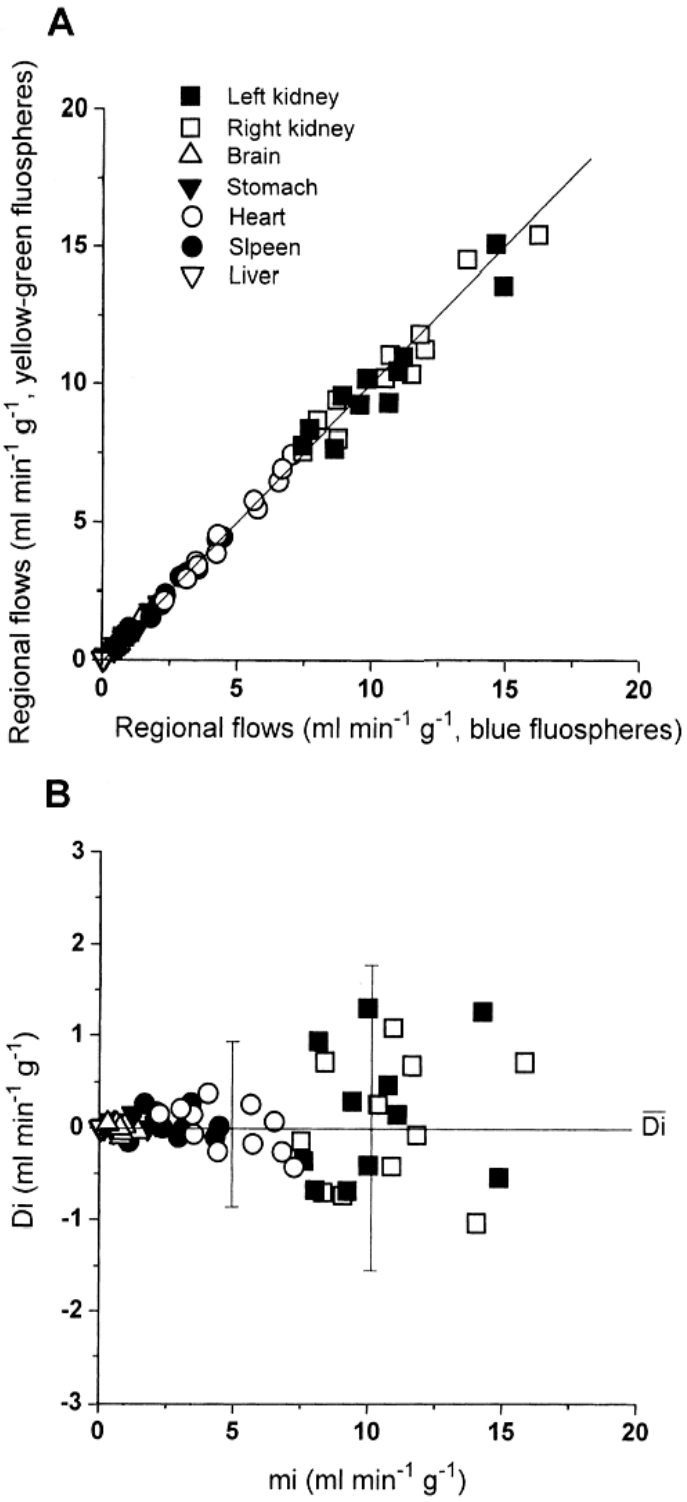

FIG. 2. **A:** Correlation of the pooled regional blood flow values measured simultaneously with yellow-green and blue fluospheres in the heart, brain, stomach, liver, spleen, and the two kidneys. **B:** Plot of the difference (D_i) between each pair of regional blood flow measurements against the mean of the pair (m_i). The horizontal line indicates the mean difference in flows, and vertical bars symbolize the 95% confidence intervals calculated for given flow values (one pair of measurements).

Extraction efficacy. **Table 3** shows that ~90% (n = 71,469 ± 991) of the total injected fluospheres (n = 79,992 ± 1,709) were trapped by the lungs (i.e., the first capillary bed encountered) when fluospheres were injected intravenously, whereas the mean number of fluospheres detected in the kidney was inferior to the fluorescence detection limit (50). As illustrated in **Fig. 3**, histologic examination of hearts and kidneys confirms that, when injected into the left ventricle, fluospheres were trapped in the microcirculation of these organs. In hearts, fluospheres are present in the interstitial capillaries but not in the arterioles, venules, or larger vessels (**Fig. 3A**). In kidneys, fluospheres are found at the end of the afferent arterioles (i.e., at the origin of the glomerular capillaries), but not in the postglomerular circulation (**Fig. 3B**).

	Lungs		Left kidney	
Animal	% Injected dose	Number of spheres	% Injected dose	Number of spheres[a]
1	90.4	72,286	0.05	<50
2	90.6	72,438	0.02	<50
3	85.8	68,666	0.03	<50
4	84.1	67,264	0.02	<50
5	92.1	73,698	0.03	<50
6	92.5	74,035	0.01	<50
7	89.1	71,242	0.03	<50
8	94.7	75,780	0.06	<50
9	84.8	67,812	0.04	<50
Mean ± SEM	89.3 ± 1.2	71,469 ± 991	0.03 ± 0.01	—

[a]Detection limit is 50 fluospheres.

TABLE 3. Extraction efficacy of 15-µm fluospheres injected into the superior vena cava of nine anesthetized C57BL/6 mice

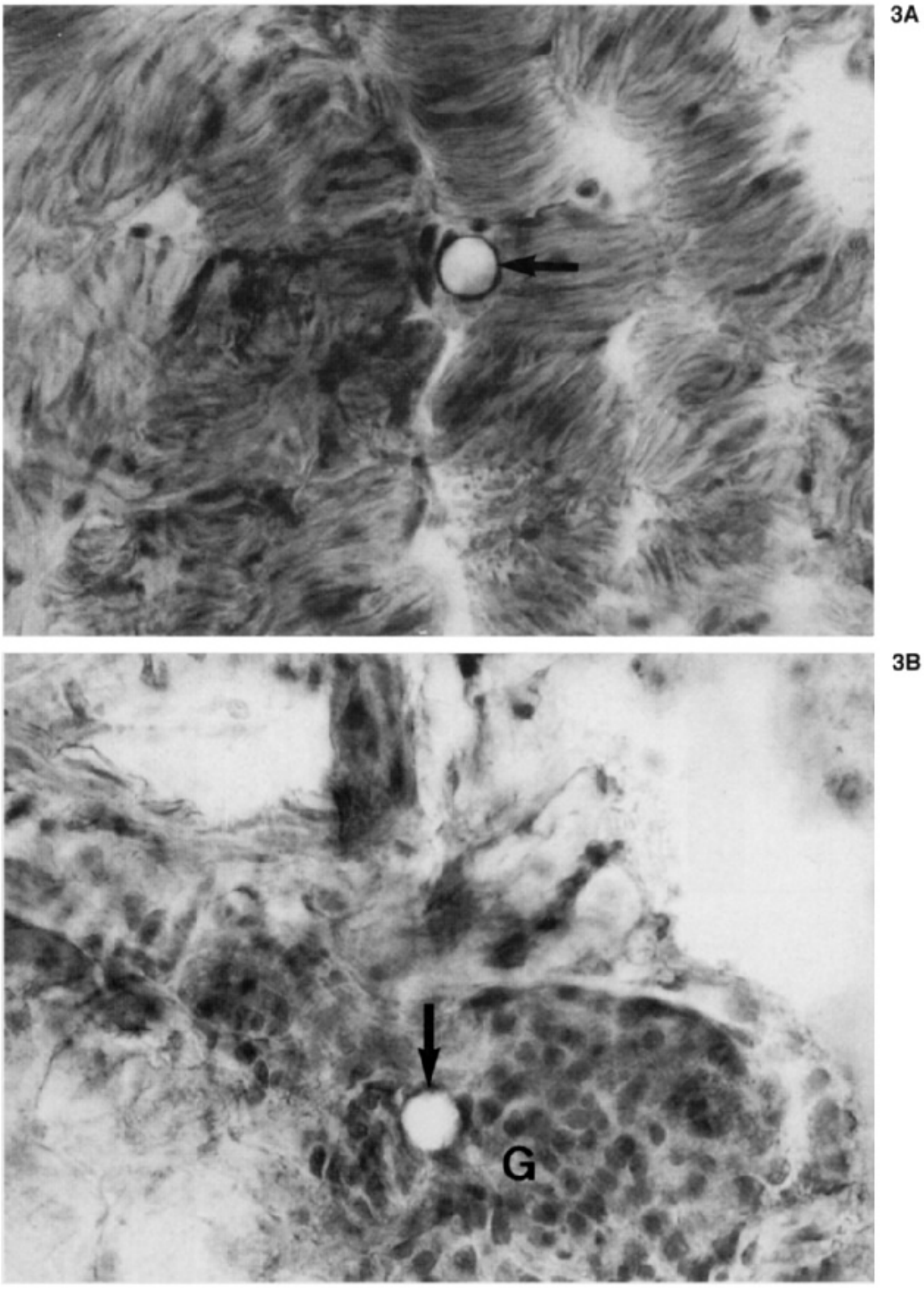

FIG. 3. Photomicrographs of a fluosphere trapped in an interstitial capillary of the heart **(A),** and in a glomerular afferent arteriole **(B).** Fluospheres are shown by arrowheads. G, glomerulus. Original magnification, ×400. Stain, hematoxylin eosin.

Systemic and regional hemodynamics in C57BL/6 mice under dynamic conditions

The cardiac and hemodynamic responses to infusions of saline, or of angiotensin II, or of increasing doses of isoprenaline in C57BL/6 anesthetized mice are shown in **Fig. 4**. As compared with saline and as expected, angiotensin II induced significant increases in blood pressure (+37%), total peripheral resistance (+95%), and coronary (+55%) and renal (+475%) vascular resistances. It also significantly decreased CO (-33%). As compared with saline, isoprenaline dose-dependently reduced blood pressure (-39% at 1 ng/g) and total peripheral resistance (-43% at 1 ng/g) and increased HR significantly (+40% at 1 ng/g). Coronary resistance was dose-dependently and significantly decreased (-62% at 1 ng/g), whereas renal resistance was dose-dependently and significantly increased (+78% at 1 ng/g).

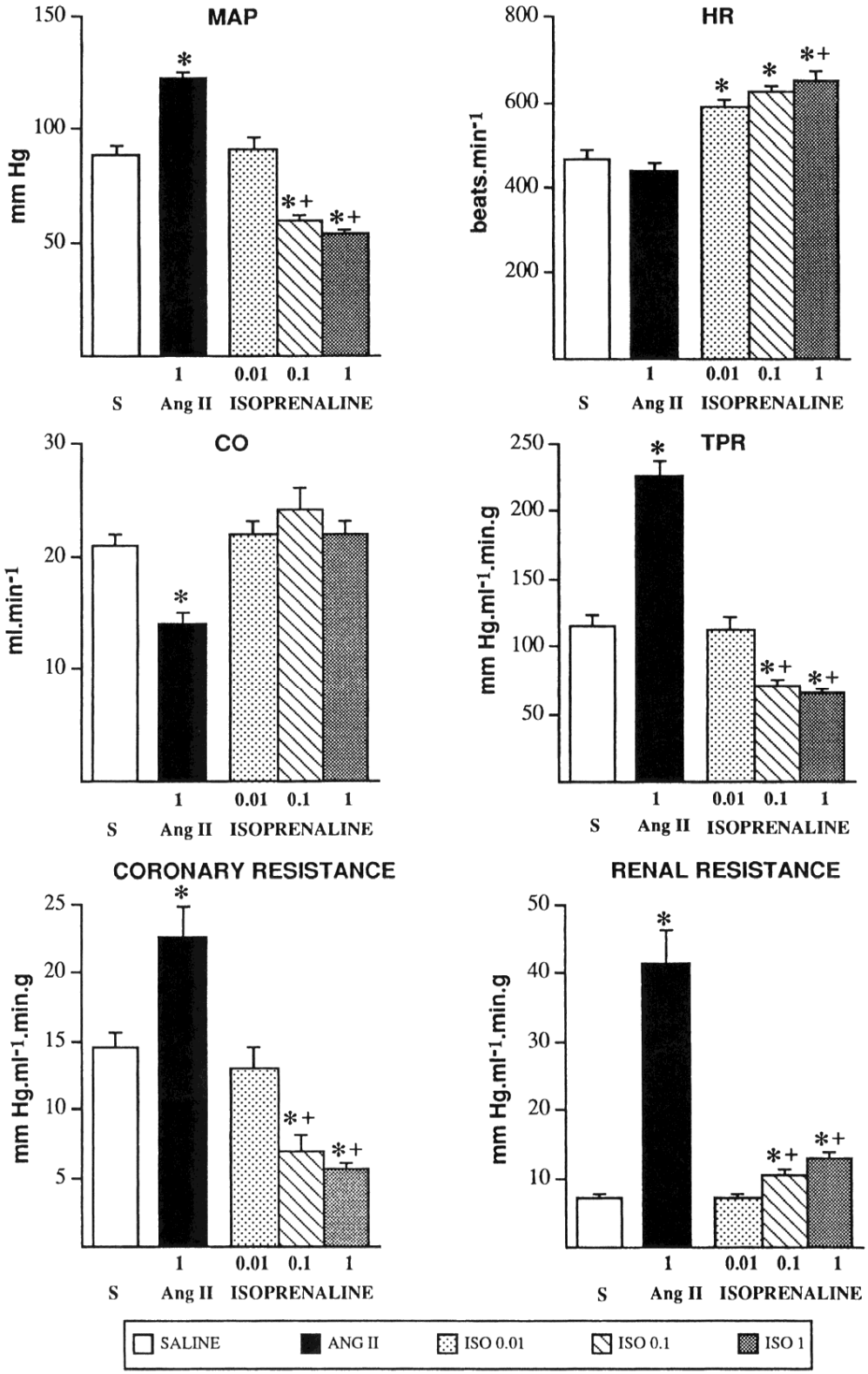

FIG. 4. Effects of intravenous infusions of saline (S), angiotensin II (Ang II, 1 ng/min · g) or isoprenaline (0.01, 0.1, 1 ng/min · g) on mean arterial pressure (MAP), heart rate (HR), cardiac output (CO), total peripheral resistance (TPR), and coronary and renal vascular resistances. *Value significantly different from S value: $p < 0.05$. †Value significantly different from corresponding isoprenaline (0.01 ng/min · g value: $p < 0.05$.

	C57BL/6	ApoE deficient
Mean arterial pressure (mm Hg)	102 ± 4	104 ± 6
Heart rate (beats/min)	485 ± 12	456 ± 14
Cardiac output (ml/min)	21.2 ± 0.7	19.4 ± 1.1
Cardiac index (ml/min · g of BW)	0.64 ± 0.03	0.58 ± 0.03
Total peripheral resistance (mm Hg · min · g BW/ml)	162 ± 9	182 ± 17
Brain		
Flow (ml/min · g)	0.48 ± 0.03	0.51 ± 0.03
Resistance (mm Hg · ml · min/g)	219 ± 15	208 ± 18
Heart		
Flow (ml/min · g)	5.6 ± 0.4	4.6 ± 0.3[a]
Resistance (mm Hg · ml · min/g)	19 ± 1	23 ± 2
Kidneys		
Flow (ml/min · g)	10.4 ± 0.7	9.6 ± 0.9
Resistance (mm Hg · ml · min/g)	10 ± 1	12 ± 2

Values expressed as mean ± SEM.
BW, body weight; kidneys, mean of the two kidneys.
[a]Value significantly different from C57BL/6 corresponding value: $p < 0.05$.

Table 4 indicates the mean ± SEM values of the systemic and regional hemodynamic parameters measured with the FM technique in 30-week-old C57BL/6 and ApoE-deficient mice.

Systemic and regional hemodynamics in ApoE-deficient mice

Regarding C57BL/6 mice, it appears from **Tables 1, 2,** and **4** that CO, regional blood flows, and corresponding regional vascular resistances were almost similar at 30 and 10 weeks of age.

Regarding 30-week-old ApoE-deficient mice, **Table 4** shows that, when compared with age-matched C57BL/6 mice, all investigated parameters were similar, except for coronary blood flow, which was significantly decreased (-18%; $p < 0.05$) and for coronary vascular resistance and total peripheral resistance, which both showed a trend to increase (+21 and +12%, respectively, both NS).

DISCUSSION

Today because of the rapidly expanding use of transgenic mice, there is an urgent need for safe, accurate, and reliable methods to investigate cardiac and systemic as well as regional hemodynamic parameters in this species. Cardiac hemodynamics and function can be assessed through ventricular catheterization, thermodilution, or echocardiographic techniques (**7-10**). Simultaneously to assess systemic and regional hemodynamics, only the microspheres technique is available. Two studies, performed with radioactive microspheres in mice (**11,12**), demonstrated the feasibility as well as the accuracy of the method at evaluating systemic and regional flows. However, radioactive microspheres have a number of disadvantages, including radiation exposure, cost of the spheres, cost for disposal of contaminated animals, etc. In this context, the main purpose of our study was to investigate whether the reference sample method using fluospheres, a technique already used in large animals (**1,2,13**) and which we recently validated in rats (**5**), could be applied to intact closed-chest mice, and especially transgenic mice, to reliably measure CO and its regional distribution.

The reference sample method using microspheres allows accurate determination of systemic and regional hemodynamics if a number of conditions are met (**12,14,15**). The technique should allow proper mixing of microspheres before reaching the aorta, adequate capillary trapping during the first circulatory pass, and no major alteration of the animals' hemodynamic status. In this study, when fluospheres were injected in the apical region of the left ventricle, proper mixing and distribution of FMs was achieved, as identical flow values were found in the left and in the right kidneys (**Tables 1 and 2**). Moreover, the simultaneous injection of two differently labeled FMs (agreement study) revealed no significant difference between the two estimations of CO and organ flows (**Figs. 1A and 2A**), there being in both cases a linear relation between these two estimations, with a slope not significantly different from unity. This indicates both that the distribution of fluospheres was the same and that there was no chance passage of groups of spheres to one stream or another. Moreover, it appears that two independent reading procedures applied to the same blood flow value do not result in significant systematic or random errors. Adequate capillary trapping of fluospheres also was achieved, as ~90% of the intravenously injected FMs were recovered in the lung and none in the kidney (**Table 3**). Moreover, histologic examination of hearts and kidneys after intraventricular injection of fluospheres clearly confirmed the capillary localization of the trapped FMs. Finally, there were no major consequences of fluospheres injection on the animals' hemodynamic status, MAP being only minimally decreased (10%), mainly because of the volume of the reference blood sample withdrawn.

CO values measured with FMs in our pentobarbitalanesthetized C57BL/6 mice (range, 18.2 ± 1.0-19.3 ± 2.1 ml/min at 10 weeks, 22-26 g BW) are very close to those previously reported in the literature for weight-matched mice and determined using (a) either radioactive microspheres in tribromoethanol-anesthetized (17.3 ± 3.1 ml/min) (11) or in conscious (18.3 ± 1.4 ml/min) C3H/HeJ mice (**12**), or (b) echocardiography in 129SvEv/Tac mice anesthetized either with pentobarbital (21 ml/min) or with a ketamine-xylazine mixture (17.5 ml/min) (**10**). These data clearly indicate that the FM technique provides CO values similar to those obtained by other methods, especially that of the reference radioactive microspheres. Furthermore, they confirm that, in mice, CO (16) and cardiac index values (**5,15,17**) are approximately one fifth of and twice, respectively, the corresponding values in rats.

Considering the distribution of CO, the regional profile we obtained with FMs in anesthetized C57BL/6 mice indicates that the brain, the heart, both kidneys, the spleen, and the stomach received 1.4-1.7%, 3.0-3.9%, 16.3-21.4%, 1.0-1.1%, and 0.8%, respectively, of CO. Slightly higher values for the brain (5%), the heart (6%), but not for the kidneys (17%), have been reported in anesthetized C3H/HeJ mice using radioactive microspheres also injected in the left ventricle (**12**), but these small discrepancies could be accounted for by the differences in the mouse strain and the nature of the anesthetic used. That slightly different coronary blood flow values could have been obtained after injection of microspheres into the left atrium cannot be excluded, but as we wished to avoid thoracotomy, the left ventricular route of injection was preferred. It should also be noticed that our regional distribution profile of CO determined with FMs in anesthetized C57BL/6 mice is very similar to that previously reported with radioactive microspheres in anesthetized rats [e.g., brain, 3%; heart, 5%; kidneys, 10%; spleen, 1%; and stomach, 1% (**16-18**)]. Finally,

regarding reproducibility, it was not possible, because of their low volemia, to withdraw from our mice two successive reference blood samples within a short period. As a result, no genuine determination of the reproducibility of the fluospheres technique could be performed in the same mouse by injecting two different sets of fluospheres 1 h apart. Nevertheless, if one compares the systemic and regional hemodynamic data obtained in the two series of experiments performed in age-matched animals and reported in **Tables 1 and 2**, it appears that the method provides identical results.

Further to validate the FM technique in mice, we also investigated its ability to assess systemic and regional hemodynamics under dynamic conditions. It is well established that angiotensin II exerts vasoconstrictor effects, especially in the kidney, and increases blood pressure, whereas isoprenaline increases heart work and hence myocardial blood flow and induces peripheral vasodilation. In this study, we were able to evidence these effects, angiotensin II increasing coronary and, to a greater extent, renal vascular resistance, whereas isoprenaline dose-dependently decreased total peripheral and coronary vascular resistances and increased HR. Interestingly, isoprenaline also dose-dependently increased renal vascular resistance, a nonclassic effect that might be explained by β-adrenoceptor stimulation-induced release of renal renin.

Finally, by using the FM method, we were able to assess the systemic and regional hemodynamic profile of 30-week-old ApoE-deficient mice and to compare it with that of age-matched C57BL/6 mice. ApoE-deficient mice appear to be hemodynamically well adapted to their gene defect at this age, as no difference was observed between the two groups of mice for any of the investigated parameters, except at the level of the coronary circulation, the first chronologically to be affected by atherosclerosis, as evidenced by the increased coronary vascular resistance and the significantly decreased coronary blood flow.

In conclusion, this study is the first to demonstrate the feasibility, the validity, the accuracy, and the reproducibility of the FM technique at evaluating systemic and regional hemodynamics in normal and/or in genetically modified anesthetized mice under both basal and dynamic conditions. It thus appears to be a useful and reliable tool to characterize cardiovascular phenotypes and investigate drugs' cardiovascular effects in genetically altered mice models.

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