

An in situ perfusion protocol of rat epididymal adipose tissue useful in metabolic studies

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Abstract Experimental approaches involving the perfusion of tissues and organs offer the advantage of improved physiological relevance over the use of isolated tissues or cells while at the same time being much more controlled and tissue-specific than studies in vivo. Nevertheless, there have been few metabolic studies performed in perfused white adipose tissue, largely because of the difficulty of the surgical technique involved. Although some methods have been described, they are difficult to use as perfusion protocols and their reproducibility is poor. We have modified a rat perfusion method, based on a modification of the Ho and Meng technique, for use with epididymal white adipose tissue (eWAT), and we present it here as a protocol to be reproduced. We also offer surgical solutions for the most common variants of vessel distributions in rats. Using the protocol described here, the perfused adipose tissue is viable and metabolically active, as indicated by the maintenance of tissue ATP levels and adiponectin secretion and by endogenous lipolysis regulation. Moreover, there is a high level of lipoprotein lipase activity in the endothelium of the tissue, which is heparin-releasable. **Thus, this method is a useful and reproducible tool that allows the perfusion of eWAT for use in metabolic studies.**—Cònsol, G., A. Moles, D. Ricart-Jané, and M. Llobera. **An in situ perfusion protocol of rat epididymal adipose tissue useful in metabolic studies.** *J. Lipid Res.* 2005. 46: 1803–1808.

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Perfusion is a technique that faithfully reproduces the physiological environment in an isolated tissue. It allows the study of metabolic processes in physiological conditions because it involves the whole organ, maintaining its structure and cellular diversity. Moreover, substrates, hormones, drugs, etc., reach cells through physiological channels, the arteries and veins.

In the early 20th century, the development of perfusion methodology in laboratory animals began in organs such

as the heart and liver. Later, some studies were performed in perfused white fat tissue. However, this technique is more complicated than in other tissues. In 1963, Robert and Scow (1) published the first perfusion of rat parametrial white fat tissue, used in later studies (1–5). In 1964, Ho and Meng (6) described a perfusion technique for isolated rat epididymal white adipose tissue (eWAT), used as a reference for subsequent epididymal tissue studies (6–10). In all cases, the protocols described are very difficult to reproduce and use in metabolic studies.

Alternatively, in humans, the use of microdialysis and arteriovenous techniques has been proposed to study white adipose tissue (WAT) metabolism (11). Although these methods are applicable to humans, they have several disadvantages, such as minimal control over the studied tissue and over the different factors released from the rest of the body.

Based on the modification by Gubser, Di Francesco, and Bickel (7) of the Ho and Meng technique (6), we developed a detailed in situ perfusion method with the aim of providing an easily reproducible protocol useful for other researchers. This method is appropriate for use in metabolic studies involving the perfusion of different substances and collection of the perfusate through a vein cannula. Also, to assess the physiological state and viability of the perfused adipose tissue, we studied the following: *a*) tissue ATP levels; *b*) adiponectin secretion; *c*) the regulation of lipolysis by epinephrine and insulin; and *d*) the release of LPL by heparin.

MATERIALS AND METHODS

Male Wistar rats (Harlan Interfauna Ibèrica, Barcelona, Spain) weighing 175–200 g were used. Animals were maintained under controlled conditions: 12/12 h light/dark cycle (lights on from 8 AM to 8 PM), temperature of $22 \pm 2^\circ\text{C}$, and humidity of $50 \pm$

Abbreviations: eWAT, epididymal white adipose tissue; WAT, white adipose tissue.

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5%. All rats were fed ad libitum with standard laboratory diet and water.

Before describing the perfusion protocol, we briefly mention some of the materials that we use to perform the perfusion because they are either essential for the method or they speed it up.

Peristaltic pump: Minipuls 2 (Gilson).

Cannulae: single lumen polyethylene tube of outer diameter 0.8 mm × inner diameter 0.5 mm (Critchley Electrical).

Krebs-Henseleit perfusion buffer at pH 7.4 containing 6.17 mM KCl, 1.54 mM KH₂PO₄, 1.58 mM MgSO₄, 25 mM NaHCO₃, 1 mM CaCl₂, 5 mM glucose, 3% (w/v) BSA, and 75 mM sodium citrate tribasic dihydrate. The perfusion buffer is preoxygenated (95% O₂, 5% CO₂) for 25–30 min and then maintained in a bath at 37°C. We do not use heparin as an anticoagulant to avoid LPL release from the endothelium (12). Sodium citrate is an effective anticoagulant and does not induce LPL release (data not shown).

Electric blanket (Daga, Barcelona, Spain) placed under the body of the rat to maintain the perfused tissue at 37°C.

Yarn used for surgery: 000 silk.

Halogen lamp: Intralux 4000-1 (Volpi).

Needle-lancet electrodes: Elektrotom 505 (Berchtold). These are useful in the finer aspects of the surgical technique but can only be used for small vessels situated far from the cava and aorta.

Perfusion protocol

The method describes the perfusion of the right epididymal fat pad, entering from the aorta and leaving through the vena cava.

We have observed a huge anatomical variability in the fine distribution of the arteries and veins of rats. The protocol that we describe attempts to explain different solutions for the most common variants that we have encountered. However, when the right spermatic vein was inserted into the vena cava below the iliolumbar vein, the animal was rejected.

The protocol is divided into six phases and 32 steps (Fig. 1) as described below.

Anesthesia

1. First, the animal is anesthetized by intraperitoneal injection of ketamine (100 mg/kg body weight) and xilacine (10 mg/kg body weight).

Precannulation

2. Make a laparotomy from the pelvis to the sternum.

3. Make two perpendicular cuts in the middle of the initial cut to facilitate the opening of the abdominal cavity. Cut the small hemorrhage from the two subcutaneous arteries with two clamps.

4. Remove the viscera from the abdominal cavity and place them to the left side of the animal.

5. Externalize the right epididymal fat and testis. Put the right epididymal fat pad over a Petri dish and maintain wet with saline at 37°C (using an electric blanket).

6. Clean the zone that surrounds the aorta and vena cava gently with fine forceps and cotton buds. After cleaning, identify the insertion of the right spermatic artery into the aorta (usually above the left renal vein). Make a ligature around the aorta above this point, but do not close it yet (an open ligature) [1]. (All numbers indicated in boldface in square brackets correspond to the ligatures shown in Fig. 2).

7. Make an open ligature around the left renal artery near its insertion into the aorta [2]. When the right spermatic artery is in-

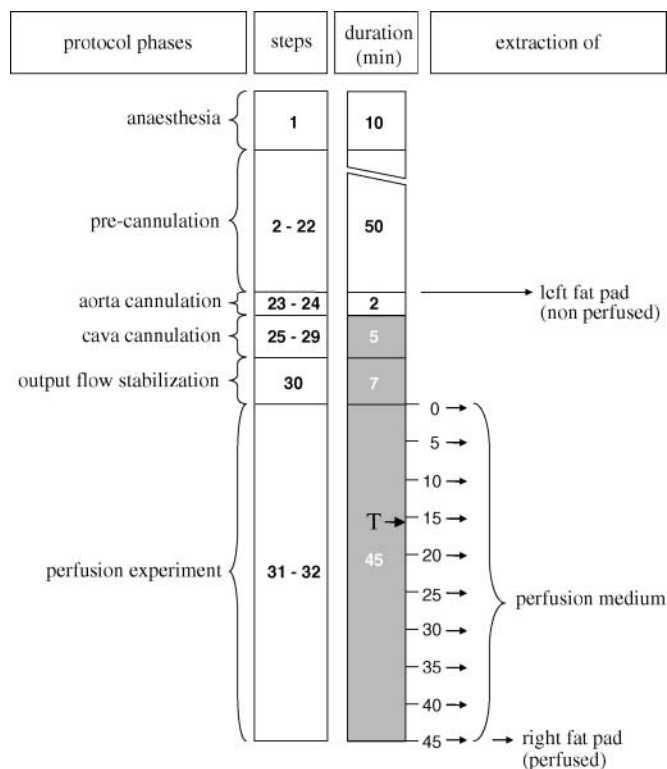


Fig. 1. Scheme of the perfusion protocol. The scheme represents the phases of the perfusion protocol, the corresponding steps, and the estimated duration (in minutes) of each phase. The gray bar indicates perfusion. From the end of the aorta cannulation phase to the end of the outflow stabilization, the tissue is washed. This phase ends when collection of the perfusion medium begins (time zero), the beginning of the experimental phase. From this point, medium is collected every 5 min. T indicates the time at which the hormones that constitute the experimental treatment (epinephrine or heparin) are added to the perfusion medium. Arrows indicate extractions (tissues and perfusion medium).

serted into the aorta below the renal artery and ligature [1] can be performed below the renal artery and above the spermatic artery, it is not necessary to perform ligature [2].

8. Separate the aorta from the vena cava between the insertion of the spermatic artery and the iliolumbar artery. There is usually a small ramification of the aorta in this zone that runs to the spinal column [3]. Tie this vessel. Sometimes the left iliolumbar artery is too close to the spermatic artery to insert the arterial cannula. In this case, tie the iliolumbar artery and separate the aorta from the vena cava below the iliolumbar artery.

9. Make an open ligature around the aorta above the insertion of the iliolumbar artery [4]. In cases in which the left iliolumbar artery is near the spermatic artery, this ligature must be performed below the iliolumbar artery.

10. Between the two open ligatures around the aorta, [1] and [4], and below the right spermatic artery insertion, make an open ligature [5] that will be used to fix the arterial cannula (see step 24).

11. Make an open ligature around the left spermatic artery as near as possible to its insertion into the aorta [6].

12. Coagulate with a lancet electrode (this is faster than with ligatures) the two ramifications of the right spermatic artery, located approximately halfway along its length [7] and [8].

13. Clean the area between the insertion point of the right spermatic vein into the vena cava and that of the right spermatic

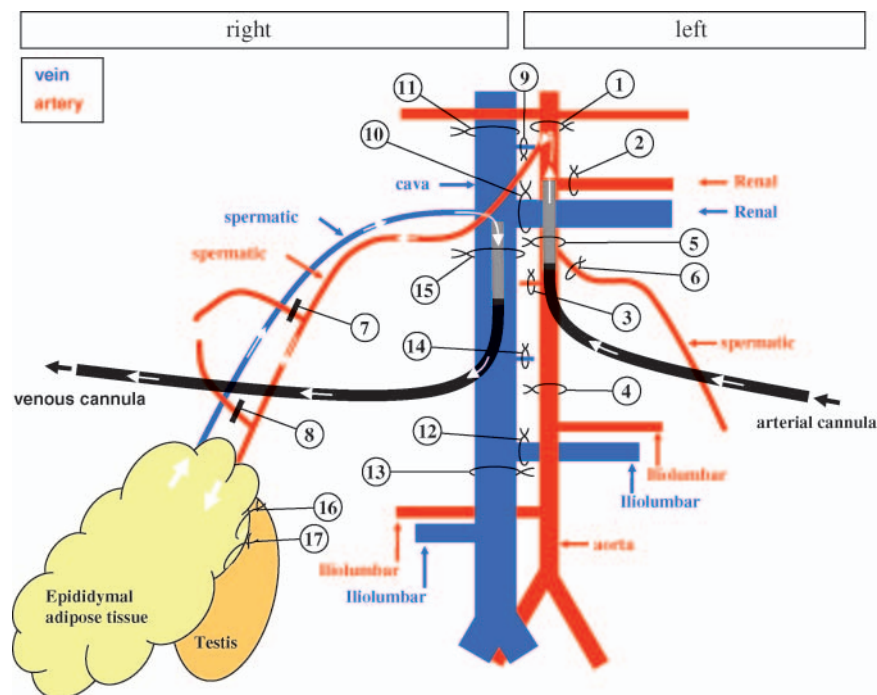


Fig. 2. Anatomical positions of ligatures and cannulations (precannulation and aorta and vena cava cannulation phases). The upper horizontal bars indicate the right and left sides of the animal. Veins are shown in blue and arteries in red. Numbers indicate the protocol step (see Materials and Methods) in order of appearance. Arterial and venous cannulae are shown in black. The region of the cannula that is inserted into the vessel is shown in gray. White arrows indicate the direction of flow.

artery into the aorta. Now, a small vein that runs to the spinal column can be identified above the left renal vein. Tie it [9].

14. Make an open ligature around the left renal vein near its insertion into the vena cava [10].

15. Make an open ligature [11] around the vena cava above both the right spermatic vein insertion and the vessel tied with ligature [9]. Some animals have sufficient distance between the insertions of the left renal vein and the right spermatic vein into the vena cava to perform ligature [11] between the two. In this case, ligature [10] is not necessary.

16. Tie the left iliolumbar vein near the vena cava [12].

17. Make an open ligature around the vena cava below the left iliolumbar and above the right iliolumbar veins [13]. If the insertions to the vena cava of the left and right iliolumbar veins are too close, open ligature [13] must be made below the right iliolumbar vein, so this vein must be tied too.

18. Usually, in the section of the vena cava between the right spermatic vein insertion and the left iliolumbar vein insertion, there are one or two small veins that run to the spinal column. They must be tied or coagulated with a lancet electrode [14].

19. Between the two open ligatures in the vena cava, [11] and [13], and below the right spermatic vein insertion, make an open ligature [15] that will be used to fix the venous cannula (see step 28).

20. Make an open ligature around the arteriovenous plexus of the right epididymal adipose tissue [16] and another around a vessel that runs between the epididymal fat pad and testis, near this plexus [17].

21. Tie ligature [6] and extract the left fat pad. This should be frozen immediately in liquid N_2 to be used as control tissue.

22. Tie ligatures [2] and [10].

Aorta cannulation

23. Tie, in this order, ligature [4] and then ligature [1].

24. Cannulate the aorta ~ 1 cm below the right spermatic artery insertion. To fix the arterial cannula into the aorta (the gray section of the arterial cannula in Fig. 1), tie open ligature [5]. A spatula placed under the aorta may be useful during the cannula implantation.

Vena cava cannulation

25. After step 24, quickly switch on the peristaltic pump (0.03–0.05 ml/min) and begin the tissue wash at $37^\circ C$ with Krebs-Henseleit buffer containing sodium citrate tribasic dihydrate as anticoagulant. We use this flow rate because, according to some authors (13, 14) and to recently submitted data from our laboratory, it represents the physiological flow rate through this fat pad. This wash should be maintained until perfusate collection begins (see step 31).

26. Tie ligatures [16] and [17].

27. Tie, in this order, ligature [11] and then ligature [13].

28. Cannulate the vena cava ~ 1 cm below the right spermatic vein insertion. To fix the venous cannula into the vena cava (gray section of the venous cannula in Fig. 1), tie open ligature [15]. As for step 24, a spatula placed under the vena cava may be useful. Place this cannula so that its distal tip is 30 cm below the level of the animal. This is done to obtain a negative pressure that will help to circulate the perfusate through the system. In addition, at the distal tip of this venous cannula, we introduce a G25 needle inserted into a 10 ml syringe, facilitating the generation of an initial gentle suction.

29. Kill the animal by opening the thoracic cavity.

Output flow stabilization

30. Washing is maintained until the volume of the efflux perfusate is stabilized (see Results).

Perfusion experiments

31. The medium was collected every 5 min in Eppendorf tubes kept in iced water. Fifteen minutes after the beginning of the collection, the perfusion medium (Krebs-Henseleit buffer at 37°C containing sodium citrate tribasic dihydrate) was changed for fresh medium containing heparin (5 U/ml) or epinephrine (10 μ M). In the insulin experiment (Fig. 3A), this hormone was present (1 nM) from the start of perfusion.

32. At the end of the perfusion period (usually 45 min), the experiment was stopped and the perfused tissue was frozen in liquid nitrogen.

Chemical determinations

LPL activity assays. Tissues were homogenized (150–200 mg/ml) in HEPES-dithiothreitol-EDTA, pH 7.5, containing heparin (5 U/ml), and LPL activity was determined according to Julve et al. (15).

LPL mass. Perfused samples were loaded on a 9% polyacrylamide gel for subsequent electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane (Bio-Rad, Segrate, Italy), followed by blotting in TBS containing 5% nonfat dry milk. Incubation with chicken IgG against bovine LPL (kindly provided by Dr. T. Olivecrona, University of Umeå, Sweden) at a dilution of 1:2,000 was performed overnight in the same buffer at 4°C. A rabbit horseradish peroxidase-labeled anti-chicken IgG (Chemicon International, Inc., Hofheim, Germany) was used at a dilution of 1:5,000 for primary antibody detection, using the Super-Signal West Pico Chemiluminescent Substrate system (Pierce, Rockford, IL). Each film was scanned and the image quantified using Phoretix software.

Glycerol. Medium was deproteinized (75:3.2 in 60% perchloric acid), and glycerol levels were determined according to the method of Garland and Randle (16), modified by the use of multiwell plates (Corning Costar) and reduced volumes of both reagents and samples (approximately one-sixth). The absorbance at 340 nm was measured with a Sunrise multiwell plate reader (Tecan).

ATP. ATP levels were determined in nonperfused (control) and 45 min perfused adipose tissues with ATP Bioluminescence Assay Kit HS II (Roche Diagnostics). Tissues were homogenized (200 mg/ml) in HEPES-dithiothreitol-EDTA buffer.

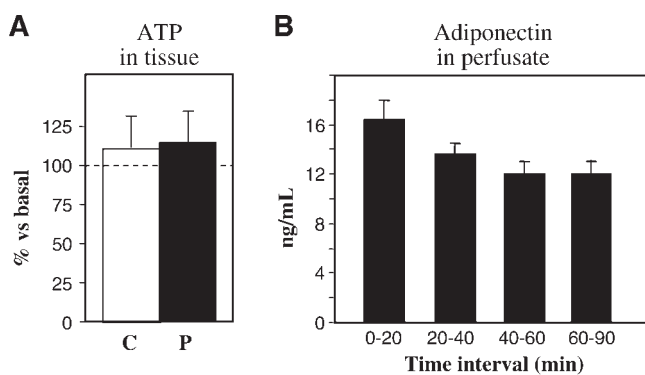


Fig. 3. ATP and adiponectin as functional indexes. A: ATP levels in 45 min perfused adipose tissue (P; black bar) and in nonperfused adipose tissue (see step 21 of perfusion protocol) (C; white bar) compared with basal levels (in adipose tissue from nonmanipulated animals; 100%). Values are means \pm SEM of six to eight animals per group. Nonsignificant differences were determined by ANOVA. B: Evolution of adiponectin release into the medium during white adipose tissue (WAT) perfusion. Values are means \pm SEM of six to eight animals in each interval. Nonsignificant differences were determined by ANOVA.

Adiponectin. Adiponectin levels in perfusates from 90 min perfused tissues were determined with the Mouse Adiponectin RIA Kit (Linco Research), which uses 125 I-labeled murine adiponectin. It is necessary to dilute the perfused samples 1:2 with distilled water before use.

RESULTS

Method validation

With an in situ preparation, the perfusion of other tissues must be excluded: nonperfused tissues did not become discolored during the perfusion. Moreover, control perfusions were performed with trypan blue to confirm that other tissues were not perfused.

The output flow (determined by measuring the amount of medium collected over a known period of time) was at least $75.8 \pm 5.4\%$ of the initial input flow. Moreover, the mean weight of the perfused fat pad was equal (0.88 ± 0.10 g) to that of the nonperfused contralateral pad (see step 21 of protocol; 0.99 ± 0.10 g). There was no visible edema as a result of perfusion.

Figure 3A shows that after 45 min of perfusion with Krebs-Henseleit medium, ATP concentration on right fat pads (perfused tissues; black bar) was equal to that on left fat pads (nonperfused tissues; white bar) and similar to that of the adipose tissue from intact animals (basal value).

Figure 3B shows that, after an initial diminution caused by slight blood contamination in the blood (the adiponectin concentration is 10^3 times higher than in the perfusate), the adiponectin concentration in the perfusate is maintained throughout the entire perfusion period (90 min).

Regulation of lipolysis by insulin and epinephrine in the perfused fat pad

Release of glycerol into the medium is a good indicator of lipolysis rate, because WAT does not contain glycerokinase and so cannot metabolize this component. Figure 4A shows that during perfusion with Krebs-Henseleit medium, the tissue exhibits an endogenous lipolysis ($0.53 \pm$

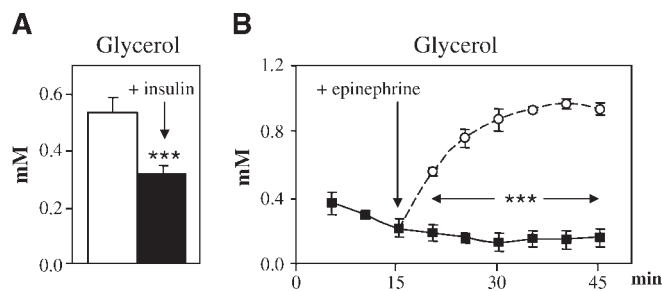


Fig. 4. Effect of insulin and epinephrine on lipolysis rate, measured by glycerol release into the perfusate. A: Glycerol release after 45 min of perfusion in the absence (white bar) or presence (black bar) of 1 nM insulin. Values are means \pm SEM of six rats, and comparisons were made by Student's *t*-test (***) $P < 0.001$. B: Time course of glycerol release into the medium during WAT perfusion. The arrow indicates epinephrine (10 μ M) addition to the perfusion medium. Values are means \pm SEM of seven rats. Statistical analysis was performed by two-way ANOVA (treatment and time) and Bonferroni's posttest (***) $P < 0.001$.

0.05 mM) that decreases to 0.31 ± 0.03 mM when the perfusion medium contains insulin (1 nM). As illustrated in Fig. 4B, 5 min after the addition of epinephrine (10 μ M), glycerol release is triggered in a time-dependent manner from the perfused fat pad. Both insulin and epinephrine effects are highly significant.

Effect of heparin on LPL activity and mass

It is well known that heparin induces the release of LPL from its endothelial anchoring (11). As illustrated in Fig. 5, the addition of heparin (5 U/ml) to the perfusion medium resulted in a 50-fold increase in LPL activity in the medium compared with non-heparin-treated perfusates ($P < 0.001$). This activity increase was accompanied by an increase in the amount of LPL protein in the perfusion medium, as detected by Western blot (Fig. 5B).

DISCUSSION

The perfusion of adipose tissue was described by Robert and Scow (1) and Ho and Meng (6) in the 1960s. Twenty years later, Gubser, Di Francesco, and Bickel (7) modified the eWAT method of Ho and Meng to perform studies on xenobiotics. Even so, this last improvement contains several information gaps that make it very difficult to reproduce the method in other laboratories. Here, we propose a variation of the Gubser, Di Francesco, and Bickel protocol for use in metabolic studies, especially of lipid metabolism, and we describe a detailed, easy-to-follow protocol. The main difficulty encountered was the huge individual variability in vessel anatomy, more apparent on the left side of the animal than on the right, making the method difficult to reproduce. However, we have succeeded in standardizing an in situ perfusion protocol for rat eWAT that is highly reproducible. This protocol is applicable to

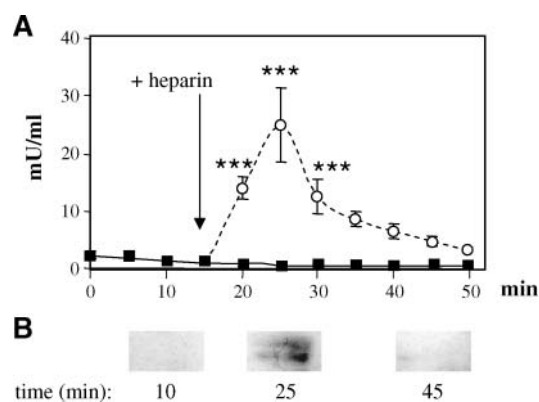


Fig. 5. Effect of heparin addition on LPL activity and protein levels in perfusate. A: The arrow indicates heparin (5 U/ml) addition to the perfusion medium. Values are means \pm SEM of six rats. Statistical analysis was performed by two-way ANOVA (treatment and time) and Bonferroni's posttest ($*** P < 0.001$). B: LPL protein in the medium was measured by Western blot with an anti-LPL antibody. Results are shown from three time points corresponding to the times indicated in A: before (left gel), 10 min after (middle gel), and 45 min after (right gel) heparin addition.

$\sim 70\%$ of male Wistar rats, because some animals must be rejected as a result of particular variations in vessel anatomy (see Materials and Methods).

Most published protocols perfuse the fat pad with defibrinated rat blood (3, 5, 17) or with Krebs-Ringer bicarbonate buffer containing 4–5% (w/v) BSA and heparin as anticoagulant (7). We have used a Krebs buffer, similar to Krebs-Ringer, containing 3% (w/v) BSA. We found that it is possible to perfuse without anticoagulant (data not shown), an opinion shared by Ho and Meng (6), although the tissue remains less clean and with some risk of plugging the vein cannula. Thus, we used citrate as an anticoagulant because, unlike heparin, it does not cause LPL release from the capillary endothelium, thereby allowing metabolic studies to be undertaken in which this enzyme is implicated.

First, we performed initial tests to ensure that the fat pad was correctly perfused.

Analysis of tissue color indicated that only the chosen fat pad was perfused, as shown by the change in color attributable to blood removal on perfusion. This was further confirmed by the addition of trypan blue to the perfusion medium; only the chosen tissue was stained by the reagent.

Perfusate losses were limited, and there was no retention of perfusion medium in the tissue, as shown by the mean weight of the perfused fat pad being equal to that of the nonperfused contralateral pad. Using the technique of Ho and Meng (6), a weight increase of 18% could be seen, and with the Gubser, Di Francesco, and Bickel modification (7), the increase was $5 \pm 7\%$. These differences in tissue weight show that the perfusate was partially retained in the tissue. On the contrary, however, with our technique, the weight is maintained and there is no visible edema attributable to the perfusion, indicating that the perfusate flows without retention.

We suggest that the reason for the liquid retention previously observed in WAT could be a flow rate that is set too high. Ho and Meng (6) used a flow rate of 0.4–0.5 ml/min, and Gubser, Di Francesco, and Bickel (7) used 0.053 ml/min/g tissue. We used a flow rate of 0.03–0.05 ml/min, previous studies having suggested this to be more physiological (our unpublished data).

We also studied whether under our experimental conditions the perfused fat pad maintains its viability and metabolic activity during the perfusion procedure. First, we measured ATP in perfused tissue because it is a typical cell viability marker (18). The ATP amount in perfused tissues was the same as in control tissues (nonperfused). This result showed that the right fat pad (perfused) was viable and metabolically active during the perfusion experiment. Also, we studied adiponectin production, because it is secreted by adipose tissue and can be considered, for our perfusion experiment, a marker of WAT activity. Our results showed that the perfused fat pad releases adiponectin constantly to the perfusate throughout the entire perfusion period (90 min).


On the other hand, we studied the lipolysis rate of the tissue arising from the endogenous reserves of triglycer-

ides. The presence of insulin and epinephrine significantly increased and decreased, respectively, basal lipolysis. These responses coincide with the known effects of these hormones and reveal that the perfused adipose tissue retains both functional receptors for these hormones and intact signal transduction pathways for the regulation of hormone-sensitive lipase activity. As an index of tissue lipolysis, we assessed glycerol concentration in the perfusate after the addition of insulin to the perfusion medium (19). As expected, insulin inhibited lipolysis. In another experiment, we added epinephrine to the perfusion medium and observed the opposite effect; that is, epinephrine stimulated lipolysis. These results confirm the data of Severson, Lefebvre, and Sloan (19) on the effect of epinephrine on glycerol release from epididymal fat pads. They also show that our method keeps the perfused WAT metabolically active.

Finally, because of the importance of LPL as a key enzyme in the lipid metabolism of WAT, we assessed the capacity of heparin to release LPL from the endothelium. In our perfusion system, heparin addition resulted in high levels of lipoprotein lipase activity in the perfused medium. This agrees with observations from other authors using white fat pads (4, 19) perfused canine subcutaneous adipose tissue (20), or isolated fat cells grown in culture (21–24).

Alternatively to perfusion, the arteriovenous method has been used in humans to study WAT metabolism (11). This technique is based on the differences measured in the concentration of a molecule between systemic arterial blood and WAT venous blood. It then becomes possible to measure the release or the uptake of molecules by this tissue.

However, the use (if possible) of drugs, hormones, radiolabeled molecules, enzymatic inhibitors/activators, or other substances is obviously limited in humans. Also, the doses used are lower because the perfusion medium runs directly to the tissue and is not diluted in the whole body circulation. On the other hand, the metabolic environment of the tissue, the influx and the efflux of molecules, and the specificity of the sample collection (with lower, if any, contamination with factors released from other tissues) are more controlled in the perfusion method. The main disadvantage of this technique is that it is limited to experimental animals.

In summary, our perfusion protocol for rat eWAT represents an improved version of the method of Gubser, Di Francesco, and Bickel (7). Moreover, we provide a step-by-step protocol designed to be used easily by other workers. Finally, our protocol allows the researcher to work with this tissue in metabolically active and cellularly viable conditions. This perfusion protocol is a powerful tool to study WAT metabolism regulation in experimental animals, because it permits the infusion of several factors directly into the tissue and the collection of samples directly and specifically from the tissue. 

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REFERENCES

1. Robert, A., and R. O. Scow. 1963. Perfusion of rat adipose tissue. *Am. J. Physiol.* **205**: 405–412.
2. Scow, R. O., F. A. Stricker, T. Y. Pick, and T. R. Clary. 1965. Effect of ACTH on FFA release and diglyceride content in perfused rat adipose tissue. *Ann. N. Y. Acad. Sci.* **131**: 288–301.
3. Chernick, S. S., R. J. Gardiner, and R. O. Scow. 1987. Restricted passage of insulin across capillary endothelium in perfused rat adipose tissue. *Am. J. Physiol.* **253**: E475–E480.
4. Blanchette-Mackie, E. J., and R. O. Scow. 1971. Sites of lipoprotein lipase activity in adipose tissue perfused with chylomicrons. *J. Cell Biol.* **51**: 1–25.
5. Rodbell, M., and R. O. Scow. 1965. Metabolism of chylomicrons and triglyceride emulsions by perfused rat adipose tissue. *Am. J. Physiol.* **208**: 106–114.
6. Ho, R. J., and H. C. Meng. 1964. A technique for the cannulation and perfusion of isolated rat epididymal fat pad. *J. Lipid Res.* **5**: 203–209.
7. Gubser, R., C. Di Francesco, and M. H. Bickel. 1986. Uptake of lipophilic model compounds into the isolated perfused rat epididymal adipose tissue. *J. Pharmacol. Exp. Ther.* **237**: 967–971.
8. Baggen, M. G. A., R. Lammers, H. Jansen, L. Verschoor, and J. C. Birkenhäger. 1987. Effects of synacthen on lipid metabolism in the perfused epididymal fat pad of the rat. *Metabolism.* **36**: 544–547.
9. Ho, S. J., R. J. Ho, and H. C. Meng. 1967. Comparison of heparin-released and epinephrine-sensitive lipases in rat adipose tissue. *Am. J. Physiol.* **212**: 284–290.
10. Kuwajima, M., D. W. Foster, and J. D. McGarry. 1988. Regulation of lipoprotein lipase in different rat tissues. *Metabolism.* **37**: 597–601.
11. Lookene, A., O. Chevreuil, P. Ostergaard, and G. Olivecrona. 1996. Interaction of lipoprotein lipase with heparin fragments and with heparan sulfate: stoichiometry, stabilization and kinetics. *Biochemistry.* **35**: 12155–12163.
12. Crandall, D. L., G. J. Hausman, and J. G. Kral. 1997. A review of the microcirculation of adipose tissue: anatomic, metabolic, and angiogenic perspectives. *Microcirculation.* **4**: 211–232.
13. Rosell, S., and E. Belfrage. 1979. Blood circulation in adipose tissue. *Physiol. Rev.* **59**: 1078–1104.
14. Summers, L. K. M., P. Arner, V. Ilic, M. L. Clark, S. M. Humphreys, and K. N. Frayn. 1998. Adipose tissue metabolism in the postprandial period: microdialysis and arteriovenous techniques compared. *Am. J. Physiol.* **274**: E651–E665.
15. Julve, J., M. Q. Robert, M. Llobera, and J. Peinado-Onsurbe. 1996. Hormonal regulation of lipoprotein lipase activity from 5-day-old rat hepatocytes. *Mol. Cell. Endocrinol.* **116**: 97–104.
16. Garland, P. B., and P. J. Randle. 1962. A rapid enzymatic assay for glycerol. *Nature.* **196**: 987–988.
17. Dauchy, R. T., D. E. Blask, and L. A. Sauer. 2000. Preparation of the inguinal fat pad for perfusion in situ in the rat: a surgical technique that preserves continuous blood flow. *Contemp. Top. Lab. Anim. Sci.* **39**: 29–33.
18. Page, R. A., K. M. Stowell, M. J. Hardman, and K. E. Kitson. 1992. The assessment of viability in isolated rat hepatocytes. *Anal. Biochem.* **200**: 171–175.
19. Severson, D. L., F. A. Lefebvre, and S. K. Sloan. 1980. Effect of chloroquine on rates of lipolysis in the isolated perfused rat heart and in rat epididymal fat pads. *J. Mol. Cell. Cardiol.* **12**: 977–992.
20. Ballard, K., B. B. Fredholm, H. C. Meng, and S. Rosell. 1971. Heparin-induced release of lipase activity from perfused canine subcutaneous adipose tissue. *Proc. Soc. Exp. Biol. Med.* **137**: 1490–1493.
21. Stewart, J. E., and M. C. Schotz. 1974. Release of lipoprotein lipase activity from isolated fat cells. *J. Biol. Chem.* **249**: 904–907.
22. Kornhauser, D. M., and M. Vaughan. 1975. Release of lipoprotein lipase from fat cells in vitro. *Biochim. Biophys. Acta.* **380**: 97–105.
23. Rothblat, G. H., and F. D. DeMartinis. 1977. Release of lipoprotein lipase from rat adipose tissue cells grown in culture. *Biochem. Biophys. Res. Commun.* **78**: 45–50.
24. Sasaki, A., and I. J. Goldberg. 1992. Lipoprotein lipase release from BFC-1 β adipocytes. *J. Biol. Chem.* **267**: 15198–15204.