I. ISOLATION OF MOUSE OR RAT PRIMARY FAT CELLS

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1). Prepare the KRH + 2.5 % BSA buffer according to the following recipe the morning of the experiment.

KRH buffer— add 200ml of water to the beaker first before the following solutions. This recipe is for a 300 ml solution.

1. 300 µl of 1M $CaCl_2$
2. 300 µl of 1.2M $MgSO_4$
3. 300 µl of 1.2M KH_2PO_4
4. 3 ml of 0.14M KCl
5. 3 ml of 0.2M pyruvic acid
6. 6 ml of 1M HEPES in 1.2M $NaHCO_3$
7. 15 ml of 2.6M NaCl
8. 7.5 g of BSA

Adjust the pH to 7.4 after the BSA has completely dissolved and bring the volume to 300 ml. Filter sterilize the solution with a 0.22 µM Cellulose Acetate filter system from Corning Inc. Place the bottle in the 37°C water bath.

2). The concentration of collagenase needed for the digestion is 1mg/ml. It is dissolved in KRH+2.5% BSA. (The current collagenase is type 4, lot number S1D4753-A from Worthington.)

3). The epidydimal fat pad is isolated from the mice(or rat) and placed in a 100 x 20 mm tissue culture dish with a small amount of KRH+2.5% BSA buffer to keep it wet. Weigh the fat pad in a weight boat (at least 1 gram of fat is needed for proper digestion per bottle with a maximum capacity of 4g/5ml of buffer). The fat is minced 30-50x with a pair of scissors. Add the minced fat to a 25ml Nalgene plastic bottle with 5 ml of 1mg/ml collagenase suspension. Parafilm and cap the bottle before putting them in the water bath to protect them from contamination. Place the bottles on their sides in a 37°C water bath and keep in place by using a rubber band. Shake the bottles for approximately 30 minutes, at 100 rpm (time of digestion varies between 30 to 45 minutes).

4). After 30 minutes of digestion, check the fat to see if it is completely digested. If the fat is not completely digested place the bottle in the 37°C water bath for a longer incubation if desired.

5). After the fat has been digested into single cells secure the 6x6 chiffon cloth by placing the cloth tightly on top of the bottle and attach a screw cap with a hole cut out of its top. Turn the bottle upside down and squeeze the bottle and cells into a 15 ml polypropylene centrifuge tube. Briefly centrifuge the cells at approximately 400 x g for 8 seconds. The cells will be floating on top of the buffer. Carefully aspirate the buffer from the bottom. Be careful not to aspirate cells from the tubes. Wash the cells 3 times

in 10 mls of KRH+2.5% BSA per wash. Transfer the cells into a 50 ml polypropylene tube and resuspend in KRH+2.5% BSA to a desired cell concentration.