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Flow-Regulated Glucose and Lipid Metabolism in Adipose Tissue, Endothelial Cell and Hepatocyte Cultures in a Modular Bioreactor

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Abbreviations:

NO :nitric oxide

MCB: Multicompartmental Bioreactor

MCmB: Multicompartmental modular Bioreactor

FFA: Free Fatty Acid

3D: 3 dimensional

PLGA:Poly (lactic-*co*-glycolic acid)

PAM: Pressure Assisted Microsyringe

CAD/CAM: Computer Aided Design/Computer Aided Manufacture

HUVEC: Human umbilical vein endothelial cell

ECGM: Endothelial Cell Growth Medium

HBSS: Hanks' Balanced Salt Solution

FBS: Fetal Bovine Serum

LFC: Laminar Flow Chamber

ANOVA: Analysis of Variance

Abstract

Static cell culture has serious limitations in its ability to represent cellular behaviour within a live organism. *In vivo*, cells are constantly exposed to the flow of bodily fluids and contact with other cell types. Bioreactors provide the opportunity to study cells in an environment that more closely resembles the *in vivo* setting because cell cultures can be exposed to dynamic flow in contact with or in proximity to other cell types.

In this study we compare the metabolic profile of a dynamic cell culture system to that of a static cell culture in three different cellular phenotypes: adipocytes, endothelial cells and hepatocytes. Albumin, glucose, free fatty acids, glycerol, and lactate were measured over 48 hours. We show that all three cell types have increased glucose uptake in the presence of flow; lactate release was also significantly affected. We provide robust evidence that the presence of flow significantly modifies cellular metabolism. While flow provides a more uniform nutrient distribution and increases metabolite turnover, our results indicate that different cell types have specific metabolic responses to flow, suggesting cell-specific flow-regulated activation of metabolite signalling pathways.

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1. Introduction

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4 Many biological processes involve dynamic interactions between cells and their
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6 microenvironment [1-3]. Mastering the ability to control these dynamic processes, *in*
7
8 *vitro*, would be potentially useful for the fabrication of tissue engineering constructs, as
9
10 well as for the study of biological processes, for example, stem cell differentiation [4].
11
12 Mechanical stimulation of cell populations helps to maintain specific cellular
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14 phenotypes and plays a significant role during differentiation and maturation of plastic
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16 cells [5, 6]. This is particularly true for engineered vascular tissue, in which *in vivo*
17
18 shear forces at the blood interface help to maintain the function of the endothelium [7,
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20 8]. Much effort has gone into the design and implementation of functional bioreactors
21
22 that mimic the *in vivo* environment. Perfusion-based culture systems have a number of
23
24 advantages over traditional static cell culture systems because complex chemical and
25
26 mechanical conditions essential to appropriate development can be better controlled.
27
28 For example, a wide variety of bioreactors for hepatocyte culture have been reported
29
30 [9,10]. Endothelial cells are also cultured in dynamic environments, usually laminar
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32 flow bioreactors, which promote cytoskeletal organization and upregulation of
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34 vasoactive factors such as NO (nitric oxide) [11]. Bioreactor culture of fat cells has not
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36 been widely investigated although two studies [12, 13] have reported that the
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38 introduction of stirring or rotation to preadipocyte cultures can induce differentiation
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40 into mature adipocytes, suggesting that mechanical forces might be important in
41
42 promoting expansion of fat cell populations. Each of these three tissues has different
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44 requirements in terms of culture conditions and maximum shear stress which they can
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46 sustain, and in fact bioreactors are often purposely designed for a specific tissue.
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57 In previous publications, we have reported on the Multicompartmental Bioreactor
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59 (MCB) [14, 15] and its modular version, the MCmB [16]. Together, hepatocytes,
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4 adipocytes, and endothelial cells form the first building blocks of an in-vitro metabolic
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6 system: hepatocytes are the main orchestrators of metabolism, endothelial cells are the
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8 highway along which metabolic products are transported [17], and adipocytes are the
9
10 sites of body fat reserves. In this study, we compare dynamic and static mono-cultures
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12 of these three tissue types by assessing cell numbers, hepatocyte albumin secretion and
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14 the concentrations of four key markers of glucose and lipid metabolism: glucose, free
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16 fatty acids (FFAs), lactate and glycerol in order to elucidate the effect of flow on cell
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18 metabolism. Our aim was to establish the differences in cell behaviour and function in
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20 the presence of flow as a precursor to studies on metabolic cross-talk in connected
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22 cultures of hepactoytes, endothelial cells and adipose tissue.
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36 **2. Materials and methods**

37 38 39 40 41 **2.1. Fabrication and treatment of scaffolds**

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43 The liver has an organized three dimensional architecture which favours cell-cell
44
45 interactions and maximizes metabolic efficiency. In order to create an optimized liver-
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47 like microenviroment for use in the bioreactor, three dimensional (3D) scaffolds of Poly
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49 (lactic-*co*-glycolic acid) (PLGA 75:25, MW 270,000; Boehringer Ingelheim, Ingelheim,
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51 Germany) were microfabricated using the PAM (Pressure Assisted Microsyringe) [18].
52
53 A liver-lobule-like design consisting of 3 layers of hexagonal elements with sides of
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55 500 μm was deposited using the rapid prototyping CAD/CAM (Computer Aided
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57 Design/Computer Aided Manufacture) features of the PAM system [19]. Overall the
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4 scaffolds have dimensions of 1 cm x 1 cm x 100 microns and can be easily handled with
5
6 tweezers and transferred in and out of microwell plates. We have shown that these
7
8 scaffolds can host high density functional cultures of HepG2 cells for up to a week [16].
9
10 Before use, the scaffolds were washed several times with sterile water, dried in an oven
11
12 at 50°C and sterilised using H₂O₂ gas-plasma available at our clinical facilities. In order
13
14 to promote cell attachment to the structures and to facilitate cell adaptation in in-vitro
15
16 culture, PureCol™ (INAMED, Leimuiden, Netherlands) a purified collagen for Tissue
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18 Engineering, cell culture and biochemistry was used at 5 µg per cm² as suggested by the
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20 manufacturer. Finally the structures were washed with PBS three times and equilibrated
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22 with fresh medium overnight in the incubator.
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30 **2.2. Cell/tissue sources**

31 Unless otherwise specified, all reagents were from Sigma-Aldrich (Sigma-Aldrich,
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33 Milan, Italy).
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36 Omental adipose tissue was obtained, with informed consent, from surgical
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38 interventions in nondiabetic subjects free of known metabolic diseases. The tissue was
39
40 chopped and partially digested with collagenase type II in HBSS (Hanks' Balanced Salt
41
42 Solution) and then added to a 200 micron mesh filter and rinsed with DMEM to remove
43
44 blood vessels. Floating partially digested adipose tissue was then divided into aliquots
45
46 and transferred to DMEM F12 20% FBS. An initial quantity of 200 mg partially
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48 digested tissue containing approximately 300,000 adipocytes was used for each
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50 condition tested.
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55 A commercially available normal human umbilical vein endothelial cell (HUVEC) line
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57 (Promocell, Heidelberg, Germany) was used to simulate the endothelial monolayer.
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4 The cells were cultured in Endothelial Cell Growth Medium (ECGM, PromoCell),
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6 composed of Endothelial Cell Basal Medium (Promocell) supplemented with 10 %
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8 FBS, 0.1 ng/ml epidermal growth factor, 1.0 ng/ml basic fibroblast growth factor, 0.4 %
9
10 endothelial cell growth supplement/heparin, 1.0 µg/ml hydrocortisone (Promocell), 100
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12 U/ml penicillin and 100 µg/ml streptomycin. In these experiments cells were used up
13
14 to the 4th passage. The source of hepatocytes was a human hepatocellular liver
15
16 carcinoma (HepG2) cell line. HepG2 cells were grown in Eagle's minimal essential
17
18 medium (EMEM, glucose 1g/liter) supplemented with 5% FBS (Fetal Bovine Serum),
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20 1% nonessential amino acids, 1% EMEM vitamins, 2 mM L-glutamine, 100 U/ml
21
22 penicillin and 100 µg/ml streptomycin. The cells were passaged using 0.05 % trypsin
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24 with 0.02% EDTA in PBS (reagents from Sigma-Aldrich) and were used up to passage
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2.3. Static Culture

All experiments were carried out using ECGM supplemented as described previously. Preliminary experiments showed that vitality of all three cell types was conserved with respect to their standard media. HUVECs were seeded on glass slides with a 12 mm diameter using 80,000 cells in 200 µl of ECGM. After 2 hours the cells adhered and at this point each slide was transferred to a 100 mm diameter petri dish with 10 ml of medium until the experiment was begun.

HepG2 cells were seeded on the PLGA scaffolds placed in 24 well microplates (BD Biosciences, Buccinasco, Italy) at a density of 100,000 cells per scaffold using 2 ml of ECGM. At 24 h the scaffolds were moved to new 24 multiwell. After a further 48 hours, when the cells had proliferated to about 250,000 cells per scaffold, each scaffold was

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4 transferred to a 100 mm diameter Petri dish. The scaffolds were then coated with an
5 alginate film consisting of 250 μ l 1% sodium alginate dissolved in serum free medium,
6
7 cross linked with 50 μ l of 1% CaCl₂. The alginate coating was used to protect the cells
8
9 from direct mechanical stress, whilst allowing adequate nutrient diffusion. The coating
10
11 also stops the scaffolds from floating off the slides [16]. After excess alginate was
12
13 removed with a pipette, 10 ml of ECGM was added to each Petri dish.
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18 Static adipose tissue culture was carried out with each sample of partially digested
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20 adipose tissue suspended in 10 ml medium in a 100 mm diameter Petri dish. For all
21
22 three monocultures, experiments were run for 15, 24, and 48 hours, respectively, at
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24 37°C and 5% CO₂. After the designated period of time, the medium was collected and
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26 stored at -80°C until metabolite measurements were taken.
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33 **2.4. MultiCompartmental modular Bioreactor (MCmB)**

34 The MCmB is a modular bioreactor system which enables different bioreactor chambers
35
36 to be connected together. It is the second generation of the Multicompartmental
37
38 Bioreactor (MCB) described in Vozzi et al. and Guzzardi et al. [14, 15] and is
39
40 composed of a variable number of cell culture chambers which can be connected in
41
42 series or parallel to create connected cultures as desired. It has a mixing chamber for
43
44 oxygenation and the addition or sampling of medium, as well as a peristaltic pump
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46 (Ismatech IPC-4, Zurich, Switerland) and tubing which connects the various cell
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48 chambers.
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53 In this work we used two different chambers. The first, MCmB 2.0, described in Vinci
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55 et al. [16], and in Mazzei et al. [20], was designed to provide extremely low shear stress
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57 to cells in culture, and to be compatible with 24-multiwell culture plates. The second,
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4 the LFC (Laminar Flow Chamber) was designed for applying uniform and laminar wall
5 shear stress to cells [21]. The LFC is a 2 mm-high parallel flow chamber made of
6 silicone. At the flow rate of 250 $\mu\text{l}/\text{min}$ used in the experiments the wall shear stress in
7 the LFC is 0.02 Pa. The wall shear stress in the cell culture region of the MCmB 2.0 at
8 the same flow rate is 10^{-5} Pa. Figure 1a illustrates the connection scheme of the
9 bioreactors and Figure 1b and c show the MCmB 2.0 and LFC.
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21 2.5. Dynamic Culture

22 The components of the MCmB were autoclaved before each use and assembled under a
23 laminar flow hood so as to connect the cell chambers with the pump, and with the
24 mixing chamber via tubing. The adipose tissue was placed in the MCmB 2.0 and 1 ml
25 medium was added to the chamber. The top of the chamber was layered with a
26 prewetted 200 micron nylon mesh sandwiched between 2-40 micron nylon mesh circles
27 in order to prevent movement of adipocytes out of the chamber and into the tubing. The
28 bioreactor circuit was filled with an additional 9 ml of medium and then connected in a
29 closed loop as shown in Figure 1a.
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42 HUVECs were seeded onto a glass coverslip as described for the static cultures and
43 allowed to adhere. The coverslip was then placed in the bottom of the LFC, and the
44 circuit filled with 10 ml of medium and closed.
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49 HepG2 cells were seeded on scaffolds and allowed to proliferate for 72 hours as
50 described. The scaffolds were transferred to the MCmB 2.0 and coated with alginate.
51 After the gel had set, the bioreactor circuit was filled with 10 ml of medium. The flow
52 rate was set to 250 $\mu\text{l}/\text{min}$ after which the bioreactor circuits were placed inside a
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37°C/5% CO₂ incubator for 15, 24, or 48 hours. After incubation, cells were observed

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4 under light and /or fluorescent microscopes to confirm cell viability and medium was
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6 collected and stored at -80°C for eventual metabolite dosing. Each condition, (cell type
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8 and time point) was run in triplicate.
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11 12 13 14 **2.6. Cell Counting and Metabolite Dosing**

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16 Adipose tissue was stained with Hoechst 33258 and observed under a fluorescent
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18 microscope (Olympus, AX70, Olympus Italia, Milan) to confirm cell viability and
19
20 absence of contamination. HUVEC and HepG2 morphology was analysed using a
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22 microscope and the total cell number for every time point was evaluated using a Burker
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24 chamber and trypan blue to exclude non-viable cells. The number of dead cells counted
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26 was always less than 2% of the total.
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30 Glycerol and D-Lactate concentrations were determined by modified Lloyd assay using
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32 an automated spectrophotometer Cobas Fara II (Roche) [22]. FFAs were measured by
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34 an enzymatic colorimetric method (NEFA C test-Wako Chemicals GmbH, Germany)
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36 and Glucose was determined by an enzymatic spectrophotometric method (Glucosio
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38 HK CP-HoribaABX, Italy).
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42 Finally albumin, one of the main markers of hepatic function, was assayed in all
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44 experiments with HepG2 cells using an enzyme-linked immunosorbent assay specific
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46 for human albumin (Bethyl Laboratories, Montgomery, TX USA).
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51 52 **2.7. Statistical analysis**

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54 Statistica Version 7 was used to carry out ANOVA, student t-tests, Mann-Whitney tests,
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56 and Bonferroni post-hoc analyses. Data were expressed as the mean \pm SD. The
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58 biological experiments were carried out in triplicate and in some cases six times. The
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4 dosing was performed in triplicate. Variations were considered to be significant if the
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6 p-value was < 0.05 . Unless noted, the p-values in the text refer to those obtained from
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8 student t-tests. Mann-Whitney analyses confirmed those obtained with t-tests in all
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10 cases.
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13 14 15 16 **3. Results** 17

18 19 20 21 **3.1 Cell Count** 22

23 Since mature adipocytes do not divide, it was assumed that the adipose tissue would not
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25 expand during the experiments. Precursor cells residing in adipose tissue can
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27 differentiate into mature adipocytes when stimulated with hormones and growth factors,
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29 including insulin and cortisol [23]. However the experimental conditions were not
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31 designed to promote such differentiation, and in any case, the differentiation process
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33 would require a longer period of time than the incubation periods studied in the present
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35 experiments. After digestion of the adipose tissue with collagenase, adipocytes with
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37 Hoechst-positive nuclei were observed using a fluorescent microscope, confirming cell
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39 viability after incubation. There was no visible change in the size or the appearance of
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41 the tissue before and after incubation and cells remained free of contamination.
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46 HUVEC and HepG2 cells maintained the same proliferation rates in static and in flow
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48 conditions, with cell counts taken before and after incubation. Our results therefore
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50 suggest that mitosis is not affected by flow or shear stress in the experimental
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52 conditions used. Furthermore, as confirmed by several studies [24] they were more
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54 elongated and oriented in the direction of flow, while in static culture they have the
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4 classic random cobblestone arrangement. No differences in morphology were noted in
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6 the HepG2 cells on the 3D scaffolds, confirming the results reported in Vinci et al [16].
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10 11 **3.2. Albumin**

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13 In dynamic culture albumin production of hepatocytes was significantly upregulated
14
15 throughout the course of the experiment when compared to the static culture. The mean
16
17 albumin concentration was over five times greater in the MCmB dynamic culture
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19 relative to the static culture (data not shown) ($p < 0.00002$).
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26 **3.3. Metabolite dosing**

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28 Table 1 summarizes the metabolite concentrations expressed as means (mM) \pm SD for
29
30 FFAs, glycerol, glucose and lactate at four time points in adipose tissue, endothelial
31
32 cells, and HepG2 cells cultured under static and flow conditions (MCmB).
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38 **3.3.1 Glucose**

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40 Both HUVEC and HepG2 cells showed significantly increased glucose uptake when
41
42 placed in dynamic conditions, as confirmed by Vozzi et al. [14] and Vinci et al. [16].
43
44 The medium glucose concentration was significantly reduced over 48H in the dynamic
45
46 HUVEC culture ($p < 0.0005$), as was that in the dynamic hepatocyte culture ($p < 0.0013$).
47
48 The adipose tissue cultures showed a small increase in glucose uptake in the dynamic
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50 condition but the change in glucose concentrations over 48H did not reach significance.
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52 None of the static culture conditions showed significant changes in glucose
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54 concentrations over time.
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3.3.2 FFAs

The dynamic HUVEC culture medium showed a significant rise in FFA concentrations over time ($p < 0.0009$) whereas both the static and dynamic HepG2 cultures showed a significant decrease in medium FFAs over time ($p < 0.007$). In the static HUVEC culture there was a small but insignificant FFA release. Both adipose tissue cultures showed net FFA release over time but neither of these conditions reached significance. The only static cell culture to show a significant change in FFAs over time was the hepatocyte culture ($p < 0.007$). The 48H decrease in medium FFAs in the dynamic hepatocyte culture was equal to that of the static hepatocyte culture, showing complete removal of FFAs from the medium over time, the only difference being that in the dynamic state all FFAs were removed from the medium by 15H whereas in the static state 48H were required. These findings suggest that the addition of dynamic flow does not change the direction of FFA movement but does change the magnitude, provoking greater FFA release in the adipose tissue and HUVEC cultures; whereas in the HepG2 culture the introduction of flow provoked a more rapid FFA uptake.

3.3.3 Glycerol

Adipose tissue culture showed significant glycerol release in both static ($p < 0.004$) and dynamic ($p < 0.001$) conditions. HepG2 culture showed glycerol uptake in both static and dynamic conditions. The change was insignificant in the static culture, whereas in the dynamic culture net uptake from the medium was significant ($p < 0.004$). The HUVEC culture showed negligible change in medium glycerol over time in the static condition but there was a small net release over time in the dynamic condition ($p < 0.03$). The simultaneous, albeit small, FFA and glycerol release from adipose tissue suggests

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4 that adipose tissue undergoes some constitutive lipolysis in the absence of hormonal
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6 stimuli.
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10 11 **3.3.4 Lactate**

12 All cultures showed a significant increase in medium lactate over time. For the adipose
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14 tissue culture, lactate release was greater in the static condition ($p < 0.02$), whereas in the
15
16 dynamic condition the increase was less pronounced ($p < 0.00008$). In the static HUVEC
17
18 culture, the rise in lactate was smaller in the static ($p < 0.003$) than in the dynamic
19
20 culture ($p < 0.0002$). In the HepG2 culture, the rise in lactate was smaller in the static
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22 culture ($p < 0.02$), whereas in the dynamic culture, lactate rose more than sevenfold with
23
24 respect to the static condition ($p < 0.000001$).
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33 **3.3.5 Static vs. Dynamic**

34 To analyze the differences between the dynamic and static cultures, we chose to focus
35
36 on the net changes or Δ values of metabolite concentrations after 48H, as after this time
37
38 cells are thought to adapt to seeding and culture conditions [25]. All three culture types
39
40 displayed a significant difference between static and dynamic conditions for the change
41
42 in glucose concentrations over 48H, with greater glucose uptake in the dynamic setting
43
44 (Fig.2). Even though the changes in glucose over time were small in the adipose tissue
45
46 cultures, the difference between static and dynamic settings did reach significance.
47
48 Adipose tissue Δ Glucose over 48H was positive in the static culture whereas it was
49
50 negative in the dynamic culture ($p < 0.009$). HUVEC Δ Glucose over 48H was also
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52 positive in the static culture while negative in the dynamic culture ($p < 0.00002$). HepG2
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4 Δ Glucose over 48H was unchanged in the static culture, whereas there was a sharp
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6 decrease in the dynamic setting ($p < 0.00003$).
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11 Although there was no significant difference between static and dynamic conditions for
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13 Δ FFAs over 48H in any of the cultures though there was a trend toward increased FFA
14
15 release in the dynamic state for both the adipose tissue and HUVEC cultures. Adipose
16
17 tissue Δ FFA over 48H was negligible but positive in the static and dynamic cultures,
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19 as was Δ FFA over 48H for the HUVEC cultures. HepG2 Δ FFA over 48H was negative
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21 in both the static and the dynamic cultures, displaying total FFA disposal in both
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23 cultures.
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31 There was no significant difference between static and dynamic conditions for
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33 Δ Glycerol over 48H in any of the cultures although mean changes in glycerol were
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35 positive in both adipose tissue cultures and negative in both HepG2 cultures. Glycerol
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37 was unchanged over 48H in the static HUVEC culture with a negligible increase in the
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39 dynamic culture.
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46 Dynamic flow induced significant increases in lactate release for HUVEC and HepG2
47
48 cultures and insignificant decrease in lactate release in adipose tissue. Adipose tissue
49
50 Δ Lactate over 48H was nonetheless positive in both static and dynamic cultures.
51
52 HUVEC Δ Lactate over 48H was 1.8-fold higher in the dynamic relative to the static
53
54 culture ($p < 0.0005$). HepG2 Δ Lactate over 48H was sevenfold higher in the dynamic
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56 relative to the static culture ($p < 0.000002$). These data demonstrate that the introduction
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4 of flow changes the magnitude of lactate release, most strikingly in the hepatocyte
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6 culture.
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10 11 **4. Discussion** 12

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14 Our aim was to establish the differences in cell behaviour and metabolism when flow is
15 introduced into a cell culture system. We used the MCmB system to meet this aim, as a
16 precursor to further studies on connected cultures of hepatocytes, endothelial cells and
17 adipose tissue. The connected culture system was designed to provide an in-vitro
18 metabolic system, and glucose, lactate, FFAs and glycerol were our main focus of
19 interest because of their relevance to the metabolic dysfunction that occurs in diabetes
20 and the metabolic syndrome. In the current monoculture studies, we have established
21 that cell proliferation rates were unaltered in the presence of flow, and therefore neither
22 a shear-induced mechanical stimulus nor the more efficient convective distribution of
23 nutrients affected mitogenesis in the experiments described.
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40 On the other hand, endothelial cells, which are known to respond strongly to flow
41 through shear stress-sensitive mechanoreceptors, completely changed their
42 morphology, becoming more elongated and aligned with the direction of flow [24].
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44 This well-known phenomenon, which has been ascribed to the reorganization of F-actin
45 and microtubule networks, is activated through mechanoreceptors. It is well known that
46 flow, especially when laminar, is of paramount importance to preserve the physiologic
47 functions of endothelial cells [26]. Alterations in flow are relevant in determining
48 plaque composition, as well as endothelial cell response to inflammation and gene
49 expression [27, 28, 29]. In the HepG2 cells, albumin, one of the main markers of
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4 endogenous hepatocyte synthetic function, was significantly upregulated, and we have
5 suggested [16], that this is due to the combined effect of enhanced transport as well as
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7 mechanical stimulation through a low velocity percolative flow which is established
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9 through the protective gel coating used in our studies.
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16 In this study we provide evidence that, in cells exposed to flow in the MCmB
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18 bioreactor, there are also notable differences in metabolism in comparison to those
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20 exposed to a static environment. In the dynamic setting, all three cell phenotypes
21
22 showed increased glucose uptake; in adipose tissue and HUVECs there was a trend
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24 towards increased FFA release while glycerol did not change (with respect to the static
25
26 conditions) in any of the cultures. Glycerol is of primary interest as the central
27
28 structural component of the major classes of biological lipids, triglycerides and
29
30 phosphatidyl phospholipids. It is also an important intermediate in carbohydrate and
31
32 lipid metabolism. In hepatocytes and adipocytes, lack of glycerol movement might be
33
34 due to a decrease of aquaporin activation, involved in glycerol membrane permeability
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36 [30, 31]. Further investigation into aquaporin expression and activity in static and
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38 dynamic settings would be necessary to test this hypothesis. In the endothelial culture
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40 the small changes in glycerol concentrations may represent a mechanism for preserving
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42 low cellular permeability, a characteristic critical to proper endothelial function [32].
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49 The application of flow had no significant effect on glycerol concentrations in any of
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51 the cultures. We also observed a positive lactate release in all cultures and the
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53 application of flow significantly increased lactate release in the HUVEC and HepG2
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55 cultures. Since proliferation rates were no different in the static and dynamic states for
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57 any of the cultures, this suggests that flow's effect on lactate is not due to enhanced
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4 proliferation. Phillips et al. report that lactate levels are a function of both glycolysis
5 and gluconeogenesis in isolated hepatocytes [33]. It is plausible that increased glucose
6 uptake in HUVEC and HepG2 promotes enhanced glycolysis, producing more lactate.
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8 Since the change in glucose uptake in adipose tissue due to flow was quite small, this
9 may explain why lactate release was not greatly enhanced in the adipose tissue with
10 flow. Although the experiments here do not allow the identification of specific
11 metabolic pathways we nevertheless show that flow is a major determinant of lactate
12 metabolism in these cells.
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15 Adipose tissue is largely dependent on insulin for its glucose uptake: since these
16 experiments were carried out in the absence of insulin, it is not surprising that the
17 changes in glucose concentrations were quite small. Nonetheless there was increased
18 glucose uptake in the dynamic state. This observation underlines the role of flow on
19 glucose metabolism within adipose tissue. The importance of the interactions between
20 blood flow and adipocytes, specifically in the light of insulin resistance, was reported
21 over two decades ago in a rodent model of obesity, the Zucker rats, in which adipocyte
22 blood flow was significantly reduced [34]. More recent data also indicate that
23 differences in adipose tissue blood flow appear relevant in terms of insulin action and
24 insulin secretion [35].
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26
27 An important finding of our study is that HUVEC and HepG2 cells took up glucose
28 only in the dynamic state. In these cells glucose uptake is not insulin-dependent, and
29 indeed, they showed much more dramatic glucose uptake than the adipose tissue. This
30 observation suggests that flow is crucial in stimulating glucose uptake, and may induce
31 the up-regulation of insulin-independent glucose transport in adipose tissue, HUVEC,
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4 and HepG2 cultures. in cell phenotypes that are not insulin dependent. The biological
5
6 relevance of this phenomenon in terms of endothelial and hepatocyte function is
7
8 presently unclear. Previous studies by Bassaneze et al. [32] have suggested that cell
9
10 surface mechanoreceptors may play an important role in coupling flow and metabolism
11
12 within cells. However this critical interaction, which has not been exploited in the
13
14 present study, should be a matter of further experiments. Several reports support this
15
16 hypothesis, for example, Yeh et al. [36] report that a constant fluid flow triggers a
17
18 cascade of events leading to glucose receptor (GLUT1) up-regulation. This process is
19
20 mediated by the phosphatidylinositol-3 kinase/Akt pathway, which can be stimulated by
21
22 shear stress [37]. Furthermore, endothelial cells exposed to fluid flow show an elevated
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24 production of the second messenger IP3, and G-protein activation which stimulate the
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26 cAMP generation and activates Ca^{2+} channels. [38, 39, 40]. All these data, taken
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28 together, may explain the increased glucose uptake observed in a dynamic system in
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30 comparison with static cell cultures. It is important to note that each individual culture
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32 type displayed a unique metabolic profile and a unique response to the introduction of
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34 flow. This suggests that, since the convective driven nutrient availability is identical in
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36 the three systems, the cells are also stimulated by shear stress in a manner which likely
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38 depends on the presence of specialised mechanoreceptors which in turn influence
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40 metabolic pathways in a cell type-dependent manner. Follow-up studies will include the
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42 screening of candidate mechanoreceptors linking flow and metabolic shifts.
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Conclusions

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4 The MCmB dynamic cell culture system is a useful tool that enables the study of
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6 applied flow through a cell culture. Furthermore, the ability to observe a dynamic cell
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8 culture system enables scientists to study cellular behaviour in an environment that
9
10 more closely resembles the *in vivo* setting than that of a static cell culture. In this study,
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12 the application of flow to cell cultures has been demonstrated to alter the metabolic
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14 profiles of adipose tissue, endothelial cells, and hepatic cells and to increase glucose
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16 uptake in all three cultures. Convective flow not only increases molecular turnover, but
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18 our results suggest this may also be accompanied by a flow-regulated activation of
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20 metabolite signalling pathways which, in turn, can alter the metabolic and functional
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22 states of cells. Culturing the three tissues in a closed loop connected culture system,
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24 could help elucidate the role of these signalling pathways in metabolic cross-talk and
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26 regulation.
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39
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43
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18 **Table and Figures legends**

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23 **Table 1.** Metabolite concentrations at four timepoints for static and MCmB cultures:
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25 adipose tissue, endothelial cells, and HepG2 cells. All concentrations are expressed in
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27 mM. Experiments were run at least in triplicate and metabolite assays were also run at
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29 least in triplicate. * $p < 0.005$, # $p < 0.05$ vs Time 0
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35 **Fig. 1a.** Connection scheme showing the closed loop fluid circuit
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40 **Fig. 1b.** Multicompartmental modular bioreactor (MCmB)
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45 **Fig. 1c.** Laminar flow chamber (LFC)
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50 **Fig. 2.** Static vs. Dynamic. Summary of the results showing changes in medium
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52 metabolites at 48H in Static vs. Dynamic (MCmB) Conditions. Asterisks denote the
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54 cultures in which there was a significant difference between static and dynamic
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56 conditions for the change in metabolite concentrations over 48H ($p < 0.05$).
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Adipose tissue

Time (hours)	Glucose				FFA				Glycerol				Lactate			
	static		MCmB		static		MCmB		static		MCmB		static		MCmB	
	means	sd	means	sd	means	sd	means	sd	means	sd	means	sd	means	sd	means	sd
0	5.27	0.42	5.27	0.42	0.039	0.014	0.039	0.014	0.069	0.009	0.069	0.009	1.79	0.12	1.79	0.12
15	5.04	0.06	5.36	0.04	0.067	0.003	0.083	0.002	0.094	0.007	0.075	0.007	2.82	0.24	2.35	0.03
24	5.28	0.00	5.21	0.07	0.037	0.002	0.079	0.004	0.075	0.010	0.081	0.010	2.13	1.07	2.82	0.07
48	5.86	0.29	5.00	0.24	0.066	0.019	0.108	0.059	0.140 *	0.012	0.134 *	0.012	4.03 *	0.85	3.05 *	0.05

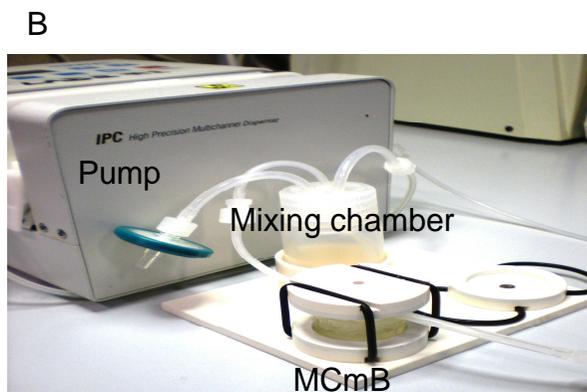
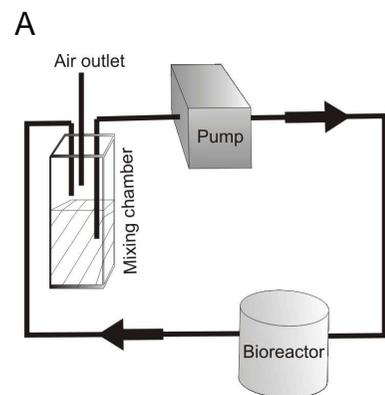
Endothelial cells

Time (hours)	Glucose				FFA				Glycerol				Lactate			
	static		MCmB		static		MCmB		static		MCmB		static		MCmB	
	means	sd	means	sd	means	sd	means	sd	means	sd	means	sd	means	sd	means	sd
0	5.27	0.42	5.27	0.42	0.039	0.014	0.039	0.014	0.069	0.009	0.069	0.009	1.79	0.12	1.79	0.12
15	5.39	0.49	4.94	0.06	0.017	0.001	0.145	0.009	0.070	0.019	0.077	0.009	2.16	0.06	2.32	0.00
24	4.85	0.09	4.78	0.00	0.019	0.003	0.132	0.003	0.080	0.023	0.068	0.009	2.41	0.05	2.63	0.10
48	5.52	0.03	2.67 *	0.06	0.059	0.023	0.114 *	0.002	0.068	0.007	0.091 *	0.007	2.31 *	0.07	2.75 *	0.03

HepG2

Time (hours)	Glucose				FFA				Glycerol				Lactate			
	static		MCmB		static		MCmB		static		MCmB		static		MCmB	
	means	sd	means	sd	means	sd	means	sd	means	sd	means	sd	means	sd	means	sd
0	5.27	0.42	5.27	0.42	0.039	0.014	0.039	0.014	0.069	0.009	0.069	0.009	1.79	0.12	1.79	0.12
15	5.14	0.03	5.02	0.18	0.020	0.002	0.000	0.000	0.059	0.002	0.060	0.003	2.09	0.26	2.60	0.27
24	5.08	0.08	4.69	0.17	0.021	0.001	0.000	0.000	0.056	0.005	0.039	0.003	1.77	0.06	3.39	0.03
48	5.28	0.19	3.28 *	0.06	0.000 #	0.000	0.000 #	0.000	0.054	0.003	0.030 *	0.003	2.30 *	0.18	5.66 *	0.08

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