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Running title: FBS affects IPEC-J2 function and bioenergetics

Abstract

The foetal bovine serum (FBS) concentration could influence functional parameters of IPEC-J2 cells. IPEC-J2 is a non-transformed continuous epithelial cell line that represents an established *in vitro* model to study porcine gut inflammation and alterations of intestinal integrity. This cell line also represents a good translational model thanks to the high similitudes between pig and human gastrointestinal tract. With the aim to assess if the FBS-dependent functional variations are linked to the bioenergetic aspects, the addition of 5% and 10% FBS in the iPEC-J2 culture medium were tested. Doubling time and TEER measurement indicated that cells cultured at higher FBS dose grow faster and as a more compact monolayer. 10% FBS increases ATP production and mitochondrial oxidative phosphorylation (OxPhos) and unaffects glycolysis. Both at 5% and 10% FBS ATP production mainly comes from OxPhos and FBS concentration does not affect the cell respiration bioenergetic parameters. Noteworthy, IPEC-J2 treated with 5% and 10% FBS have a metabolic potential since both OxPhos and glycolysis increase by >100% and <50%, respectively in comparison with baseline metabolism. Moreover, glucose, fatty acids and glutamine constitute the preferred metabolic fuel for mitochondrial respiration at both FBS conditions tested. Accordingly, the cells flexibility to oxidize these substrates shows that IPEC-J2 mitochondria cannot maintain the basal ATP production without oxidizing all the substrates available irrespective of FBS concentration. To sum up, in IPEC-J2 cells OxPhos increases with the FBS-stimulated functional physiological parameters to fulfil ATP requirements.

Keywords: IPEC-J2; cell metabolism; oxidative phosphorylation; mitochondria; glycolysis; foetal bovine serum.

1. Introduction

The IPEC-J2 cell lines, isolated from pig small intestine, are neither tumorigenic nor transformed and show morphological and functional similarity to the porcine enterocyte. This cell line represents a well-established model to simulate the human intestinal barrier by mimicking the human physiology more closely than any other cell line of non-human origin (Vergauwen 2015). IPEC-J2 show epithelial phenotypic characteristic: they grow as polarized monolayer made compact by the lateral expression of tight junction's proteins as occludin and claudin (Mariani et al. 2009). Their apical side is equipped with microvilli and they produce mucin recreating the in vivo protective mucous layer (Vergauwen 2015). Moreover, IPEC-J2 express several members of the Toll-like receptor family important molecules for the recognition of pathogen components, and if stimulated they can produce cytokines and chemokines (Schierack et al. 2006). Overall IPEC-J2 cells, represent a favourable *in vitro* model for research applications, being an ideal tool to study epithelial transport, interaction with enteric pathogens, as well as the effects of probiotics and nutrients. IPEC-J2 has been successfully cultured using many different protocols (Vergauwen 2015), in which serum, mostly foetal bovine serum (FBS), has been added to provide a wide variety of nutrients, carrier proteins, hormones attachment and growth factors. Moreover, it has been demonstrated that the amount and the type of serum added to the culture medium of IPEC-J2 cells induces a process of spontaneous differentiation that modifies the functional parameters involved in the formation of a polarized monolayer with low or high transepithelial electrical resistance (TEER) (Zakrzewski et al. 2013). While IPEC-J2 cells have been largely studied for their nutrient requirements and membrane transport features (Mao et al. 2018; Zuo et al. 2019), as far as we are aware the energy metabolism of these cells has been poorly explored. Cellular metabolism is a flexible network that allows tissues to meet demands for homeostasis and growth. The metabolic shift from oxidative phosphorylation (OxPhos) to glycolysis under oxygen availability is known as Warburg effect (DeBerardinis and Chandel 2020). Cells with a glycolytic phenotype exhibit significantly higher rates of proton production by glucose fermentation to lactate (detected as extracellular acidification rate, ECAR) than cells using OxPhos (detected as oxygen consumption rate, OCR). Bioenergetic profiling is currently used to assess the amount of energy in the form of ATP produced by mitochondrial versus non-mitochondrial respiration, namely respiration (oxygen consumption) which yields ATP synthesis, and uncoupled respiration, which does not produce ATP, by adding to the chamber containing cells a series of metabolic inhibitors (Shum et al. 2016). Shifts in substrate utilization and energy metabolism can be conveniently and simultaneously detected by evaluating the OCR, to quantify mitochondrial respiration, and the ECAR, an indicator of glycolysis. In general, glycolytic dependence, which is a common metabolic feature of stem and cancer cells, may also represent an environmental adaptation strategy to maintain the cell identity during proliferation (Yuan et al. 2019). Conversely, during differentiation a phenotypic and functional change in bioenergetics due to the related cell energy requirements is featured by an increase in mitochondrial OxPhos (Nesci 2017). It is essential to consider the epithelial cell biology in the light of the two metabolic pathways of ATP production in the cells, namely glycolysis and oxidative phosphorylation (OxPhos), being the latter, in terms of energy, much more efficient. Accordingly, the energy-consuming transport processes along the epithelial barrier of the gastrointestinal tract requires an efficient oxygen turnover whose extent depends on the nutrient availability (Vaugelade et al. 1994). In intestinal cells, in general oxygen consumption stimulates cellular activity, but the physiological activation depends on nutrient absorption. Since information on IPEC-J2 bioenergetics is scanty, in this work we aim at ascertaining if different concentrations of fetal bovine serum (FBS), which provides nutrients to the cultured IPEC-J2, can modify the IPEC-J2 functional properties and energy metabolism. The results, obtained in a well-established cell model, can contribute to cast light on the physiological metabolic changes in enterocytes during nutrient absorption.

2. Material and methods

2.1. Cell culture

The non-transformed cell line IPEC-J2 (intestinal porcine epithelial cells from jejunum) was purchased from the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH" (DSMZ). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (4.5 g/L glucose) added with 10% or 5% of foetal bovine serum (FBS, Life Technologies) and 1x antibiotic-antimycotic solution (Life Technologies) in a 5% CO_2 atmosphere at 37°C. Cultures were split weekly in 25 or 75 cm^2 culture flasks (Corning-Becton Dickinson and Company Becton Drive, Franklin Lakes, NJ, USA). The doubling time (DT) was calculated as indicated previously (Zaniboni et al. 2014), briefly DT = h/CD, where h was the culture time in hours between two passages and CD was the cell doubling calculated as CD = $(\log_{10}N - \log_{10}N_0)/\log_{10}2$, N is the number of cells at 80–90% confluency and N_0 is the number of cells seeded The monolayer formation and integrity were assessed through the measurement of trans-epithelial electrical resistance (TEER). Briefly, cells were seeded into transparent PET membrane (0.4 µm pore size) inserts in 24 well plates at a density of 1×10^5 cells/well. TEER measurements were conducted daily, using a Millicell ERS-2 Voltohmmeter® (Millipore, Billerica, MA) and the values were expressed as Ohm × cm^2 (Ωcm^2).

2.2. Cellular Bioenergetics

The Seahorse XFp analyzer (Agilent) was used to simultaneously measure oxygen consumption rate (OCR), an index of cell respiration (pmoL/min), and extracellular acidification rate (ECAR), an index of glycolysis (mpH/min). IPEC-J2 cells (10k/well) were grown in XFp cell culture miniplates (Agilent) for 24 hours. On the experiment day, IPEC-J2 were switched to freshly made Seahorse XF DMEM medium pH 7.4 supplied with 10 mM glucose, 1 mM sodium pyruvate, and 2 mM L-glutamine. The plates were incubated at 37°C in air for 45' before measuring OCR and ECAR by the adequate programs (ATP Rate Assay, Cell Mito Stress Test and Cell Energy Phenotype Test). The injection ports of XFp sensor cartridges, which were hydrated overnight with XF calibrant at 37 °C, were loaded with 10x concentration of inhibitors according to the instructions provided by Seahorse XFp ATP Rate Assay, Cell Mito Stress Test and Cell Energy Phenotype Test. The final concentration used for ATP Rate Assay were 1.5 µM oligomycin (port A) and 0.5 µM rotenone (Rot) plus antimycin A (AA) (port B). For Cell Mito Stress Test the final concentrations were 1.5 µM oligomycin (olig) (port A), 1.0 µM Carbonyl-cyanide-4-(trifluoromethoxy) phenyhydrazone (FCCP) (port B) and 0.5 µM rotenone plus antimycin A (port C), while for the Cell Energy Phenotype Test the final concentrations were 1.5 µM oligomycin plus 1.0 µM FCCP (port A). All the analysis were run at 37°C. All data were analyzed by WAVE software version 2.6.1; OCR and ECAR values were normalized to the total number of cells per each well. All parameter values were calculated per well according to the manufacturer's instructions. Both ATP Rate Assay, Mito Stress Test and Cell Energy Phenotype Test were carried out three times on different days.

The ATP Rate Assay provides the bioenergetic parameters currently used to characterize the cellular ATP production, namely ATP production rate, related to the conversion of glucose to lactate in the glycolytic pathway (glycoATP Production Rate) and to mitochondrial OxPhos (mitoATP Production Rate). Accordingly, the ratio between mitoATP Production Rate and glycoATP Production Rate (ATP Rate Index) is currently considered as a valuable parameter to detect changes and/or differences in the metabolic phenotype (a ratio > 1 means mainly OxPhos pathway; ratio < 1 means mainly glycolytic pathway).

The Mito Stress Test enables to characterize cell respiration by the following parameters: basal respiration, detected as baseline OCR before oligomycin addition; minimal respiration measured as OCR in the presence of oligomycin; maximal respiration evaluated as OCR after FCCP addition. The so-called proton leak which corresponds to the difference between the basal respiration and the respiration in the presence of oligomycin (minimal respiration), indicates the re-entry of H^+ in the intermembrane space independently of the F_1F_O-ATP synthase. The non-mitochondrial respiration, evaluated as OCR in the presence of rotenone plus antimycin A (respiratory chain inhibitors), was subtracted from all the above parameters. The ATP turnover or oligomycin-sensitive respiration was obtained from the difference between the basal respiration and the

minimal respiration (OCR in presence of oligomycin). Finally, the difference between the maximal and the basal respiration provided the spare capacity, which represents the ability to respond to an increased energy demand and can be considered as a measure of the flexibility of the OxPhos machinery.

The simultaneous measurement of mitochondrial respiration and glycolysis was carried out by the Cell Energy Phenotype Test under baseline and stressed conditions, the latter after simultaneous addition of oligomycin and FCCP. Oligomycin inhibits the mitochondrial ATP production by the F_1F_O-ATPsynthase and the cell compensates the failed OxPhos by increasing the glycolysis rate, while the dissipation of electrochemical gradient of H^+ in mitochondria by the ionophore FCCP drives the highest oxygen consumption (uncoupled respiration). The assay allows to evaluate two main parameters of cell energy metabolism known as metabolic phenotypes (baseline and stressed phenotype) and metabolic potential. The baseline phenotype is featured by the OCR and ECAR values in cells under the starting condition in the presence of substrates. The stressed phenotype is shown by the OCR and ECAR values in cells after addition of stressor compounds (oligomycin plus FCCP). The metabolic potential is the ability to increase energy production via respiration and glycolysis and it is defined as the % increase of stressed phenotype over baseline phenotype of OCR and ECAR.

The Fuel Flex Test determines the rate of oxidation of glucose (Glu), fatty acids (FA), and glutamine (Gln) by measuring OCR. Accordingly, the decline in oxygen consumption mirrors the decrease in fuel oxidation. The parameters cell Dependency, Capacity, and Flexibility to oxidize the three mitochondrial fuels was evaluated according to the protocol of the Fuel Flex Test kit, by blocking the nutrient oxidation pathways by specific inhibitors. By sequentially inhibiting the pathway of interest, followed by the two alternative pathways, this method allows to calculate how the cells exploit the pathway of interest to fulfil their energy demand. According to the protocol adopted, the wells contained the final concentrations of 3.0 µM BPTES (Bis-2-(5-phenylacetamido- 1,3,4-thiadiazol—2-yl)ethyl sulfide), allosteric inhibitor of glutamine oxidase which blocks glutamine oxidation pathway, 4.0 µM etomoxir, the long chain fatty acid pathway inhibitor, which inhibits the carnitine palmitoyl-transferase 1A coding gene, or 2.0 µM UK5099, which inhibits glucose oxidation by blocking the mitochondrial pyruvate carrier. The fuel Dependency represents the cell reliance on a stated oxidative pathway to maintain baseline respiration. It is calculated by adding the specific inhibitor to block the pathway of interest followed by the inhibitors of two alternative pathways. Conversely, the fuel Capacity is shown by the OCR driven by a specific substrate oxidation, obtained by blocking the other fuel pathways. The fuel Flexibility was obtained by subtracting the fuel Dependency from the fuel Capacity, for each pathway under study; this parameter describes the mitochondrial ability to switch from one oxidative pathway to another to allow OCR maintenance when a specific pathway is inhibited.

2.3. Statistical Analysis

Statistical analyses were performed by SIGMASTAT software. Each treatment was replicated three or eight times (viability test) in three independent experiments. Data were analyzed by the Student's t-test, or by one-way analysis of variance (ANOVA) followed by Students–Newman–Keuls' test when *F* values indicated significance ($P \leq 0.05$) was applied. Percentage data were arcsin-transformed before statistical analyses to ensure normality.

3. Results

3.1. Functional parameters: doubling time and TEER Measurement

IPEC-J2 grew adherently as epithelial monolayer both cultured at 5% or 10% of FBS (Fig. 1a). The different percentage of serum determined differences in the doubling time, in fact the mean of DT calculated for ten

generations showed that 10% of serum determined a significative decrease of the IPEC-J2 duplication time (Fig. 1b). IPEC-J2 cells were seeded at a high density for TEER measurements and formed a compact monolayer 24h after cell seeding, so no cell proliferation was required before starting TEER measurements. The different percentage of serum impacted also on TEER. The measurement of TEER began to rise already two days after the cells seeding on transwell, reaching the maximum at about 7 days post-seeding. Then TEER remained stable for 3 days before to decline. Even if the trend of TEER is the same both for cells cultured at 5 or 10% FBS, TEER values reached with the highest serum concentration were always higher than those obtained with the lowes one. Furthermore, the downward trend of TEER in IPEC-J2 cultured at 5% respect to 10% was faster.

3.2. Intracellular ATP levels

The ATP rate production is obtained from OCR and ECAR values under basal conditions. Injection of oligomycin inhibits mitochondrial ATP synthesis, while the subsequent addition of rotenone plus antimycin A causes strong inhibition of mitochondrial respiration. Thus, the depicted kinetic profile of OCR and ECAR measurements data allow the calculation of the mitoATP and glycoATP production rate (Fig. 2a). IPEC-J2 cultured for 24 h at 5% and 10% FBS concentration show a different total ATP production rate; the 10% FBS promotes a higher amount of cellular ATP than the 5% FBS due to the OxPhos increase while glycolysis is unaffected (Fig. 2a). On considering the total ATP production, IPEC-J2 cells at 5% and 10% FBS show an ATP rate index greater than 1, which points out in both cases a prevailing mitochondrial oxidative metabolism with respect to the glycolytic pathway (Fig. 2b). The doubling time results show an increased cell growth rate in the presence of high FBS concentrations, which are consistent with the increased ATP production (Fig. 2c). However, glycolysis does not increase ATP production when cell doubling time is lower at 10% FBS than 5%FBS (Fig. 2d). Since the slope (σ) of the straight line obtained (Fig. 2c) is of the same magnitude order as that of the total ATP production (Fig. 2e), namely 1.585 vs 1.452, the mitochondrial ATP synthesis is the main responsible for the energy production required by cell proliferation. Moreover, the energy metabolism detected in IPEC-J2 cultured for 96 h show a different profile with a decreased ATP production with respect to that detected after 24 h from cell seeding. Moreover, 5% FBS IPEC-J2 show a higher ATP production than the 10% FBS ones, even if this difference cannot be ascribed to a preferential higher contribution of OxPhos or glycolysis (Fig. S1a). Irrespective of FBS concentration, the ATP rate index is lower than 1, thus pointing out that, under these experimental conditions, IPEC-J2 cells mainly rely on the glycolytic pathway (Fig. S1b). Mitochondrial ATP production of IPEC-J2 cell cultured in 5% and 10% FBS 96 h after seeding, is significantly decreased and energy metabolism mainly rely on glycolysis, an effect ascribed to the confluence of the cell culture and irrespective of FBS concentration.

3.3. Cell respiration

The mitochondrial respiration expressed as OCR of IPEC-J2 cells grown at different concentrations of FBS is shown in figure 3a. The kinetic profile obtained by using serially injected mitochondrial inhibitors (olig, FCCP, Rot+AA), is not substantially different at the two FBS concentrations tested. Indeed, bioenergetic parameters do not show any significant change at the two FBS concentrations (Fig. 3b). However, the OCR values of basal respiration and ATP turnover confirm a satisfactory coupling efficiency with 0.73±0.03 a.u. at 5% FBS and 0.69±0.05 a.u. at 10% FBS. The spare respiratory capacity, which defines the cell propensity to adjust cell bioenergetics to fulfil the increased energy demand required by proliferation, represents 63.1% and 56.4% of the maximal respiration at 5% and 10% FBS, respectively. These results are consistent with the striking increase in the maximal respiration, evaluated after FCCP addition, which attains more than twofold higher values than basal respiration.

On considering the energy production mode, namely mitochondrial OxPhos and glycolysis, the metabolic switch between these two modes can be detected in a single pairwise experiment under the two FBS conditions to point out the metabolic phenotype of IPEC-J2 (Fig. 3c). IPEC-J2 at starting assay condition (baseline phenotype) in the presence of substrates and without stressor compounds (oligomycin plus FCCP) do not show any difference in the metabolic pathways at the two FBS concentrations tested. Conversely, the stressed metabolic phenotype evaluated in the presence of stressor compounds, only shows a slightly higher mitochondrial respiration in 5% FBS treated cells than in 10% FBS treated ones (Fig. 3c). There is no difference in stressed ECAR between 5% and 10% FBS treated IPEC-J2. However, the metabolic potential of IPEC-J2, which are able to increase ATP production in response to the cell energy demand, primarily lies in the efficient mitochondrial energy metabolism in both FBS conditions tested. Indeed, the metabolic potential of OxPhos increases by 131.7% at 5%FBS and by 104.3% at 10% FBS, while glycolysis only increases by 46.0% and 41.3% at 5% and 10% FBS, respectively compared to baseline metabolism. Moreover, 5% FBS-treated IPEC-J2 are able to increase their aerobic potential more than 10% FBS treated IPEC-J2 when cellular ATP production is required, while no difference in ECAR between the two differently FBS-treated cells under stressed conditions is detectable (Fig. 3d).

In order to evaluate if the different FBS concentration (5% and 10% FBS) may affect the preferred substrate utilization for mitochondrial respiration in 24 h culture IPEC-J2, as we proved that confluent cells mainly rely on glycolysis for ATP production (Fig. S1). The parameters fuel Dependency, Capacity, and Flexibility of IPEC-J2 cells for the oxidation of glucose, glutamine and fatty acids were evaluated by sequential injections of the inhibitors of each oxidative pathway, as described in Section 2.2 (Fig. 4). IPEC-J2 cells show a higher Dependency for glutamine oxidation at 5% FBS than at 10% FBS (Fig. 4a), whereas the Capacity and Flexibility do not show any difference between the two differently FBS treated IPEC-J2. On considering the oxidation of FAs, 5% FBS IPEC-J2 show higher Dependency and Flexibility and a lower Capacity than 10% FBS IPEC-J2 cells. (Fig. 4b). Finally, the only difference in glucose oxidation between the two differently FBS-treated IPEC-J2 consists in the higher Capacity at 10% FBS than at 5% FBS (Fig. 4c).

4. Discussion

IPEC-J2 cells represent a unique tool for investigating porcine intestinal barrier function and an excellent translational model, in fact they mimic human intestinal barrier more closely than other tumor cell lines and non-human cell lines (Vergauwen 2015; van der Hee et al. 2020). Many different culture conditions have been tested since their first isolation in 1989 and it has been demonstrated that the percentage of serum used has an impact on functional parameters (Vergauwen 2015). In the present research, we confirmed that higher percentage of serum determined a decrease of the time of duplication, cells grew faster. Furthermore, even if in both culture condition cell grew as an epithelial monolayer (Geens and Niewold 2011), IPEC-J2 at 10% FBS created a more compact junctions as indicated by the TEER measurement conducted for seventeen days.

The two FBS concentrations tested affect the IPEC-J2 cell functional parameters and the energy metabolism adapts to satisfy the physiological demands of the cell (Kaelin and McKnight 2013). Indeed, the IPEC-J2 cells cultured at the higher FBS concentration increase ATP production by enhancing the mitochondrial OxPhos without changing the glycolytic ATP synthesis, which remains constant under the two conditions tested (Fig. 2a). Thus, IPEC-J2 cells mainly rely on mitochondrial oxidation that accounts for the production of more than 50% of cellular ATP (Fig. 2b). These results are consistent with the stimulation of cell growth by 10% FBS (Fig. 1b). Indeed, MitoATP and total ATP production rates, but not glycoATP production rate, are increased by the 10% FBS treatment which also stimulates IPEC-J2 proliferation as shown by the decrease in the doubling time (Fig. 2c-e). The relationship between cell growth and bioenergetics allows to better understand the role of energy metabolism in IPEC-J2 proliferation and represents a metabolic index which indicates how much these

cells rely on mitoATP production, in other words on OxPhos. Therefore, the results strengthen the role of mitochondrial OxPhos as main energy supplier for IPEC-J2 cell growth, even if a change in the metabolic profile occurs when the cells are confluent and prefer glycolysis to mitochondrial respiration (Fig. S1). However, the increased ATP production provided by mitochondria in the 10% FBS treated IPEC-J2 is not associated with changes in the mitochondrial bioenergetic parameters (Fig. 3a,b) (Zhou et al. 2017). A possible key role in the modification of cellular metabolism, in response to the adaptation of the IPEC-J2 physiology to the change in FBS concentrations, is probably played by changes in biosignalling and/or gene expression (Hüttemann et al. 2007; Villena 2015). Moreover, if IPEC-J2 cells are under stressed conditions and cells require a higher energy supply, mitochondria are the main source of cellular ATP production (Fig. 3c). Moreover, the OxPhos stimulation, which is more evident in 5% FBS treated cells, (Fig. 3d) and has the meaning to supply energy to the cell, while glycolysis is apparently unaffected, mirrors the cell ability to synthesize more ATP molecules at a lower bioenergetic cost (Balaban 1990; Kim and Dang 2005). Mitochondria can utilize three different nutrients, *i.e.* glucose, fatty acids, and glutamine, to power the IPEC-J2 respiration. Each nutrient type is essential to maintain the basal OCR. When fuel Dependency overwhelms Capacity for a stated fuel, it is clear that that the fuel under study is required and oxidized to maintain basal OCR (Fig. 4). Indeed, the inhibition of one substrate pathway cannot be offset by using alternative fuel pathway(s) as the Flexibility has a negative value irrespective of the substrate. While no differences in Flexibility are detected between 5% and 10% FBS IPEC-J2 cells with both glutamine and glucose as substrates (Fig. 4a,c), apparently 5% FBS favors ATP production via mitochondrial FA oxidation. Accordingly, the contribution of mitochondrial FA oxidation to ATP production in IPEC-J2 cells is higher when cells are cultured in 5% FBS than in 10% FBS (Fig. 4b). Most likely, since FA oxidation is much more efficient than the other two pathways in terms of ATP production, the lower FBS concentration addresses IPEC-J2 cells to exploit this advantageous pathway.

On balance, the present findings which point out the capability of IPEC-J2 cells to adapt their functional physiological parameters in response to different FBS concentrations, highlight that the oxidative energy metabolism can be remodeled to fulfill the energy demands of the cell without altering the mitochondrial bioenergetic parameters. Due to the close similarity between swine and human intestinal function, research on IPEC-J2 cells may provide useful information not only on the physiology and energy metabolism of enterocytes, but also on the relevant role of environmental stimuli in addressing cell bioenergetics.

Conflict of interests

The authors declare that there are no conflict of interests.

Authorship

C.B. and S.N. conceived and designed the experiments; C.A. and D.L.M. performed the experiments; A.Z., R.S. and F.T. resources and formal analysis; S.N. analyzed the data; C.B., M.F., A.P. and S.N. supervised the experiments; C.B., M.F., A.P. and S.N. wrote the manuscript; M.F. and A.P. revised the text; S.N. funding acquisition. All authors read and approved the manuscript.

Acknowledgements

This work was supported by the CARISBO Foundation Grant n° 2019.0534 to S.N., Bologna, Italy.

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Figure

Figure 1. Effect of different FBS concentrations on IPEC-J2 functional parameters. a) Representative images of IPEC-J2 cultured at 5 or 10 % of FBS: no evident morphology differences were detected. Scale bar 100 µm b) Doubling time of IPEC-J2 cultured at different % of FBS: cells cultured at 10% FBS showed significative decrease in the duplication time. Data represent the mean ± SD *P≤ 0.05. c) Time course of transepithelial electrical resistence (TEER) measurement of IPEC-J2 cultured at different FBS percentages: cells cultured at the higher dose showed higher TEER value compared to cells cultured at the lower dose. Data represent the mean ± SD *P≤0.05 **P≤0.01 ***P≤0.001.

Figure 2. ATP production in IPEC-J2 cells. ~~a~~) Quantification of ATP production by mitochondrial oxidative phosphorylation ( ) or by the glycolytic pathway (glucose conversion to lactate) ( ). b) The plot shows the ratio between the mitochondrial ATP production rate and glycolytic ATP production rate (logarithmic scale). c) total ATP production rate (d) glycoATP production rate and (e) mitoATP production rate linked to the doubling times of IPEC-J2 at 5% (●) and 10% (●) FBS. Data are expressed as column chart (a plot) and points (b,c,e, and e plots) which represent the mean ± SD (vertical bars) from three experiments carried out on different cell preparations. * indicates significant differences (P≤0.05).

Figure 3. Energy profile of IPEC-J2 cells cultured at 5% and 10% FBS. a) Oxygen consumption rate (OCR) at 5% (●) and 10% (●) FBS under basal conditions and after the addition of 1.5 µM oligomycin (olig), 1.0 µM FCCP and a mixture of 0.5 µM rotenone plus antimycin A (rot+AA) by injections indicated by the dotted lines. b) Mitochondrial parameters (basal respiration, ATP production, proton leak, maximal respiration, spare respiratory capacity, non-mitochondrial oxygen consumption, ATP turnover) in 5% ( ) and 10% ( ) FBS-treated IPEC-J2. c) Metabolic phenotype of IPEC-J2. Baseline phenotype of 5% FBS (open blue circle) and 10% FBS-treated IPEC-J2 (open orange square) and stressed phenotype of 5% FBS (closed blue circle) and 10% FBS-treated IPEC-J2 (closed orange square). D) Metabolic potential in “Stressed OCR” and “Stressed ECAR”, expressed as % “Baseline OCR” and “Baseline ECAR” (dashed horizontal line), in 5% ( ) and 10% ( ) FBS treated IPEC-J2. Data expressed as points (~~a~~,c) and column chart (b,d) represent the mean ± SD (vertical and where present also horizontal bars) from three experiments carried out on different cell preparations. * indicates significant differences (P≤0.05).

Figure 4. Determination of the rate of fuels oxidation by measuring IPEC-J2 respiration. Fuel Dependency, Capacity and Flexibility are calculated on oxidation of glucose (a), fatty acids (b), and glutamine (c). All three fuel parameters (Dependency, Capacity, and Flexibility) are displayed as a bar chart for 5% ( ) and 10% ( ) FBS treated IPEC-J2. Gln, glutamine; FA, fatty acidis; Glu, glucose. The column chart represent the mean ± SD (vertical bars) from three experiments carried out on different cell preparations. * indicates significant differences (P≤0.05).

Figure 1

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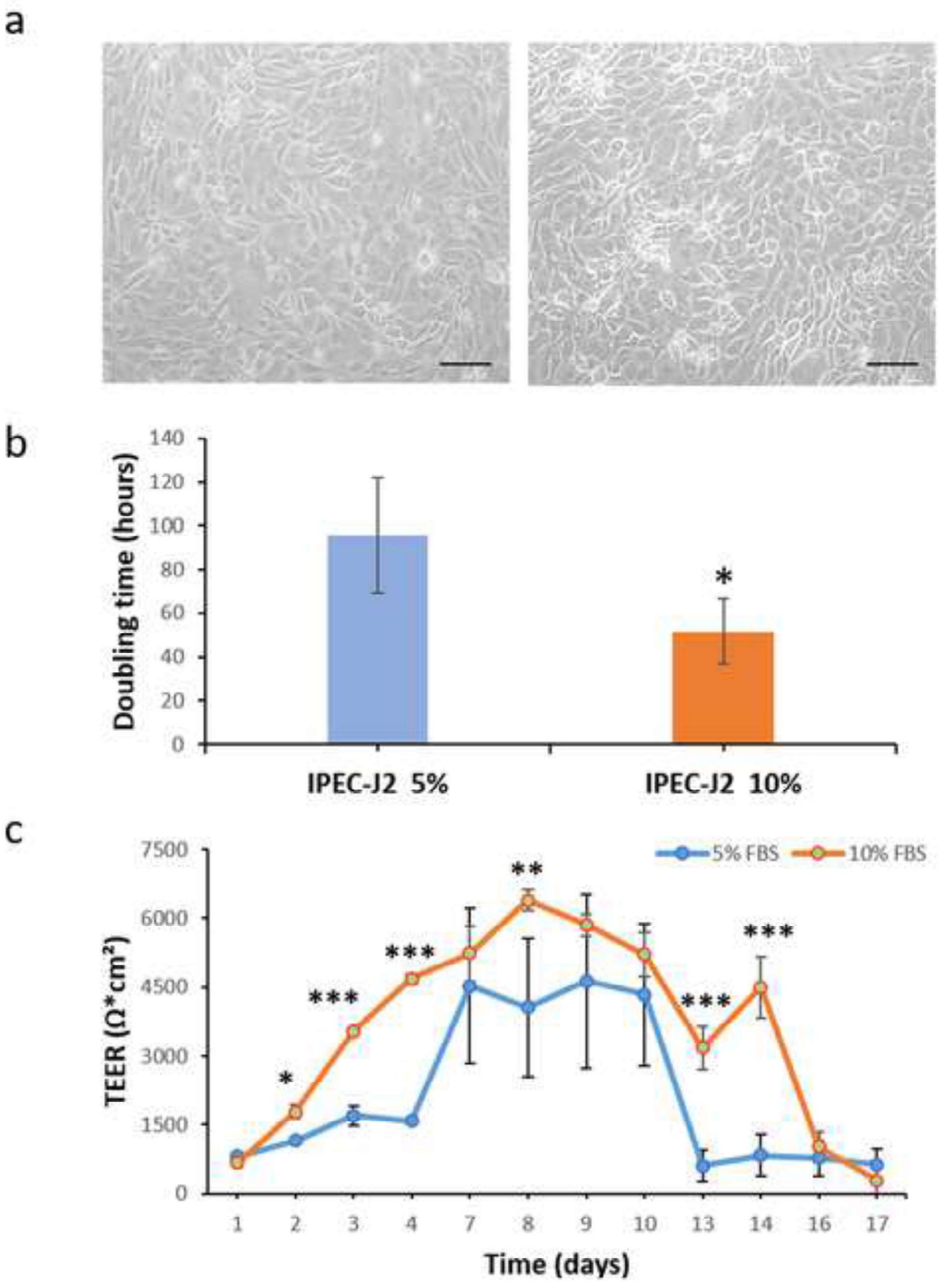

Figure 2

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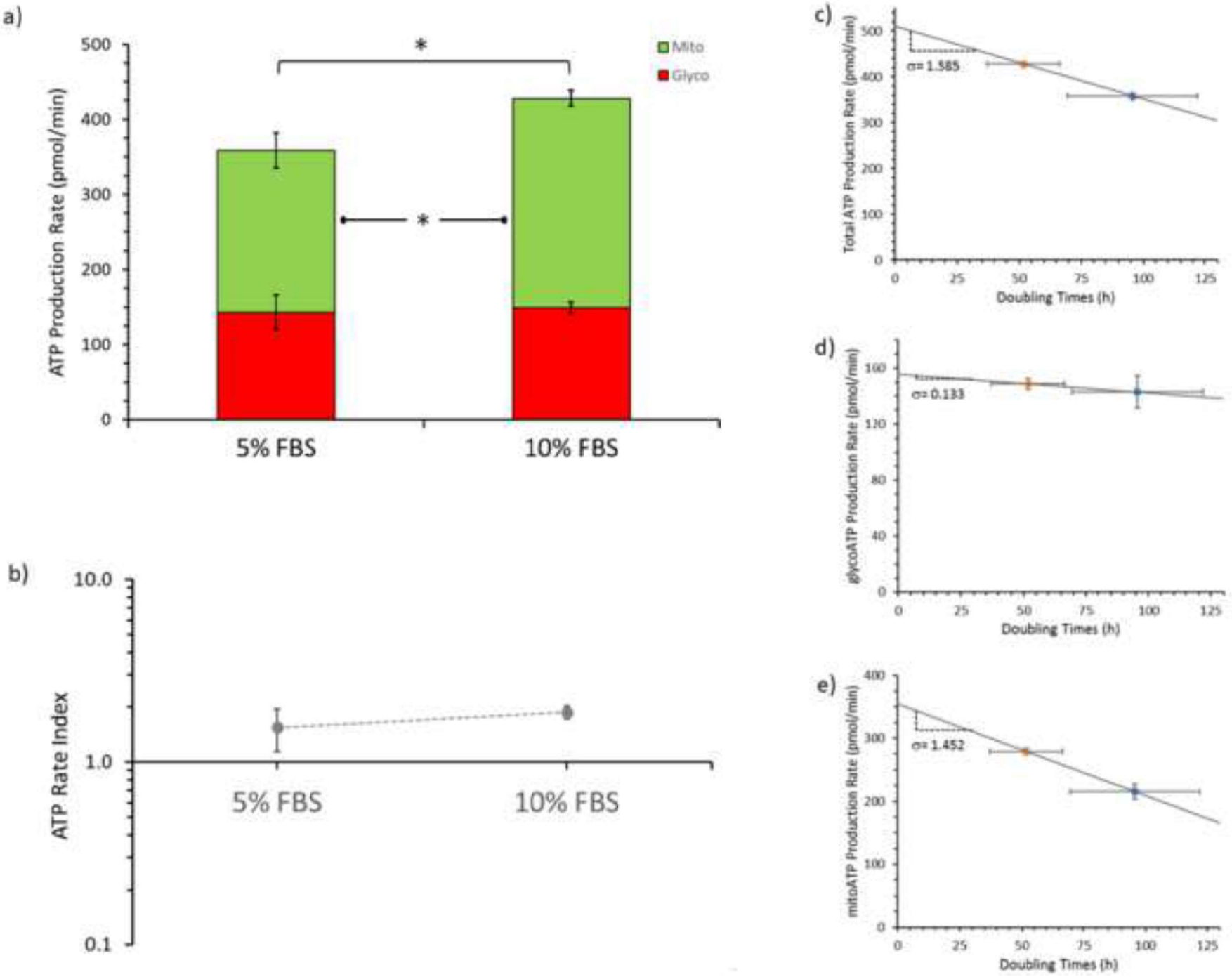

Figure 3

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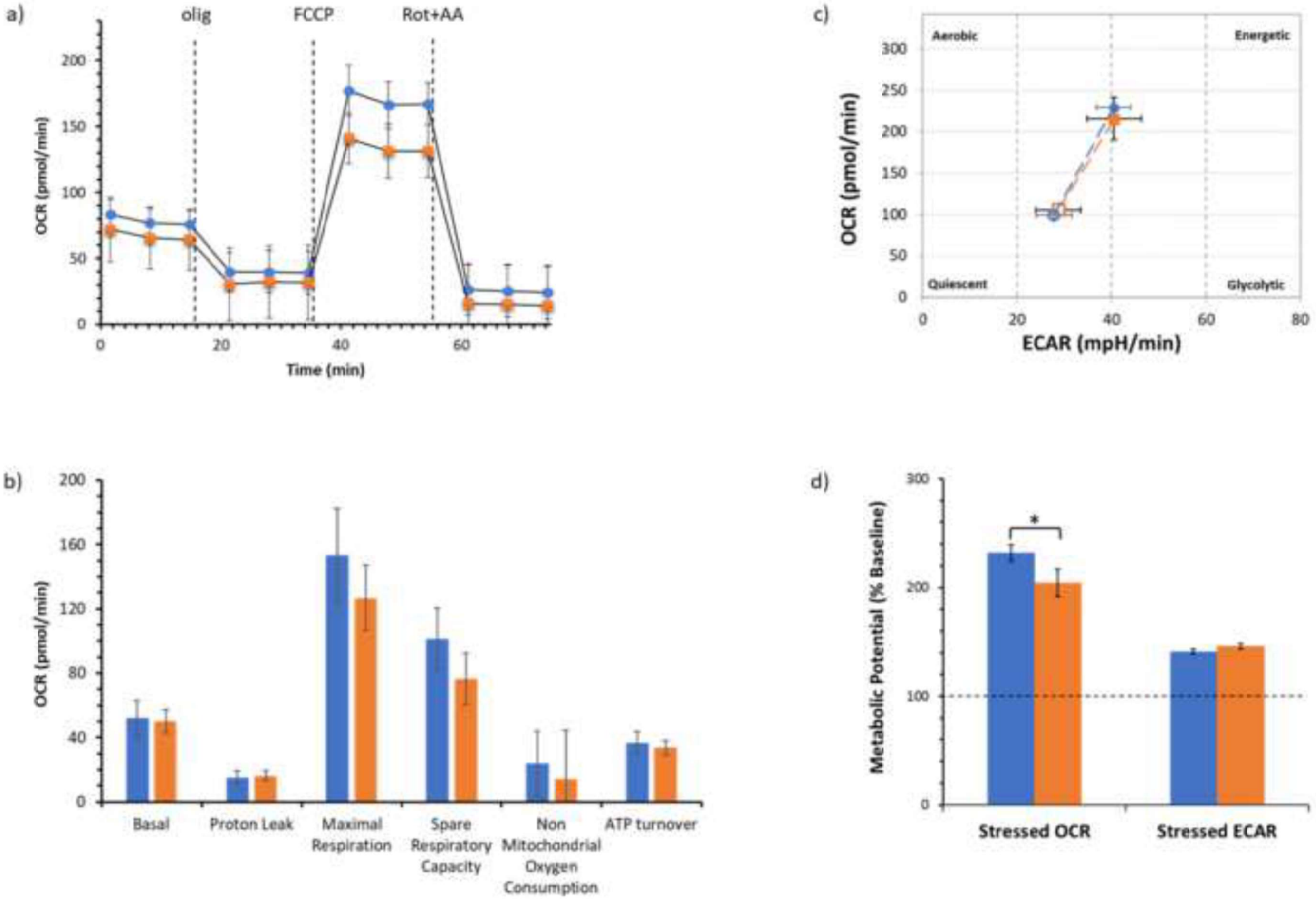

Figure 4

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