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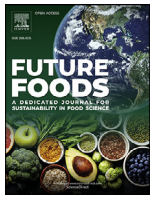

The effect of serum-free media on the metabolic yields and growth rates of C2C12 cells in the context of cultivated meat production

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ABSTRACT

Successful commercialization of cultivated meat products as alternative sources of dietary protein for a growing global population will require development of efficient and inexpensive serum-free cell culture media. It is known that serum-free media elicit dissimilar cell growth behavior compared to serum-based media, but data comprehensively exploring the effects of serum-free culture on the nutrient requirements of cultivated meat-relevant cell types have yet to be reported. We performed spent media analysis of C2C12 cells growing in Essential 8 serum-free media and in conventional serum-containing media. Data indicated that although the cell growth rates were similar in the two media over seven days, there were significant differences in the utilization rates of some key nutrients such as glucose, glutamine, glycine, and cystine. We extrapolated from the data that roughly 250–275 g of amino acids and 1100–1500 g of glucose would be required to produce 1 kg of C2C12 cells, though these requirements may change when using more optimized cells, media, and feed strategies. Our results highlight the influence of serum and serum replacements on cell metabolism, and indicate that previous data from metabolic studies performed using serum-containing media may not directly translate to serum-free systems.

1. Introduction

Cell culture media formulation remains a key technical hurdle to successful commercialization of cultivated meat (CM) at scale (O'Neill et al., 2021; Post et al., 2020). There are several design challenges unique to CM media that are not present in other applications of cell culture media. Namely, the formulations should be animal product-free to align with consumers' ethical expectations. Most importantly, CM media will need to be substantially cheaper than all existing commercial animal cell culture media to permit price parity of CM products with conventional meat (Negulescu et al., 2023; Risner et al., 2020).

Animal sera—especially fetal bovine serum (FBS)—are frequently used in animal cell culture media as rich and relatively convenient sources of crucial growth factors and other trace nutrients necessary to support cell growth *in vitro*. However, the use of serum in CM media is antithetical to the overall goals of the CM industry: FBS has several important drawbacks in regard to consistency, economics, and ethics, which have been reviewed extensively by us and others (Brindley et al., 2012; Even et al., 2006; Khodabukus and Baar, 2014; O'Neill et al., 2021; van der Valk et al., 2018). Many companies and academics in the CM space still use FBS in early research and development work since cost effective serum-free media (SFM) do not exist. This fact highlights the continued pressing need for foundational research into how to inexpensively replace animal serum. It also raises the important question of whether the media and bioprocess design optimization work performed using serum-based formulations can be directly translated to the serum-free formulations that will ultimately be most relevant for CM (Fujita et al., 2010; Lawson and Purslow, 2000; Tan et al., 2015).

A recent focus of our work has been to elucidate broad trends in the specific media requirements of cell types important to the CM industry via spent medium analysis (SMA). We have demonstrated using chicken muscle cells, chicken fibroblasts, and murine C2C12 muscle cells grown

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in serum-containing media that there are significant differences between the cell types in the cellular utilization rates of several key nutrients including glucose, arginine, glutamine, isoleucine, leucine, methionine, serine, and basic fibroblast growth factor (FGF2) (O'Neill et al., 2022). The differences in these utilization rates appeared to be most significant in the earliest stages of the cell culture period. Interestingly, most other amino acids, vitamins, and minerals tested did not appear to be depleted by the cells over time, indicating possible targets for media optimization to reduce costs.

The main conclusion we have been able to draw from our work so far has been that there are indeed differences in the nutrient needs of CM-relevant cells from different species, as well as from different lineages within the same species. This would suggest that significant effort may be required to optimize media formulations specific to each of the cell types of interest for CM production. To build upon this basic assumption, though, it remains to be demonstrated whether the same key nutrient requirements will exist in a serum-free system. That is, does the presence or absence of serum influence nutrient metabolism of CM-relevant cells? In the present study we compared key nutrient utilization patterns and cell growth rates of C2C12 cells grown in a medium with 20% FBS versus Essential 8^{TM} (E8) serum-free media.

2. Methods

2.1. General cell culture

C2C12 (CRL-1772^{TM}) (American Type Culture Collection, Manassas, VA) cells were cultured under the following general conditions. A period of expansion (proliferation) was first performed to obtain enough cells to inoculate the spent media collection experiments. The medium for this expansion (PCGM) consisted of 40% Dulbecco's Modified Eagle Medium (DMEM) (cat. # 11,995,073, Gibco, Waltham, MA), 40% Ham's F10 Nutrient Mix (Gibco cat. # 11,550,043), 20% FBS (Gibco), and additional 2.5 ng/mL recombinant human basic fibroblast growth factor (Cell Signaling Technology, Danvers, MA). Cells were maintained on tissue culture-treated (TC) polystyrene dishes, seeded at 5000 cells per cm^2 and passaged upon reaching about 75% confluence using TrypLE Express dissociation reagent (Gibco). Counting was performed via hemacytometer using trypan blue exclusion. Cultures were maintained at 37 °C and 5% CO_2 in humidified incubators throughout the experimental period.

2.2. Experimental media collection

Six-well TC-treated polystyrene plates were coated with Matrigel (growth factor-reduced, cat. # 356,231, Corning, Corning, NY), using a conventional thin-coating protocol with a diluted solution at 0.15 mg/mL of protein. Sufficient wells were prepared to allow for three replicate wells per experimental media type per predetermined collection time points. C2C12 cells at passage 10 were resuspended in either serum-free Essential 8 (Gibco) or PCGM media and seeded in their respective sets of wells at 10^5 cells in a total volume of exactly 3 mL of media per well. Table 1 provides a comparison of the chemical compositions of the two media formulations. 2 μL of a concentrated stock solution of Hoechst 33,342 nuclear stain (ThermoFisher, Waltham, MA) was added to each well shortly thereafter to give a final concentration of 0.2 μg/mL in the wells. After cell seeding, the 6-well plates were left undisturbed in the cell culture incubator, with no media changes, until the predetermined collection times. At these time points, the corresponding set of three replicate wells from both media groups were imaged using an ImageXpress Pico high content screening microscope system (Molecular Devices, San Jose, CA). The instrument's software, CellReporterXpress v2.9, was used to obtain cell counts based on nuclear stain image segmentation. After imaging, all the media were collected from the three replicate wells from each media; the collected media

Table 1
A comparison of the formulations of the primary cell growth medium (PCGM) and Essential 8^{TM} media.

Component	Concentration (mg/L)	
	PCGM*	E8**
Amino Acids		
Glycine	15	18.75
L-Alanine	3.6	4.45
L-Arginine hydrochloride	118	147.5
L-Asparagine-H2O	6	7.5
L-Aspartic acid	5.2	6.65
L-Cysteine	35.2	17.56
L-Cystine 2HCl		31.29
L-Glutamic Acid	5.88	7.35
L-Glutamine	292	365
L-Histidine hydrochloride-H2O	26	31.48
L-Isoleucine	43.04	54.47
L-Leucine	47.2	59.05
L-Lysine hydrochloride	70	91.25
L-Methionine	13.8	17.24
L-Phenylalanine	28.4	35.48
L-Proline	4.6	17.25
L-Serine	21	26.25
L-Threonine	39.44	53.45
L-Tryptophan	6.64	9.02
L-Tyrosine disodium salt dihydrate	42.648	55.79
L-Valine	39	52.85
Vitamins		
Biotin	0.0096	0.0035
Choline chloride	1.88	8.98
D-Calcium pantothenate	1.88	2.24
Folic Acid	2.12	2.65
Niacinamide	1.84	2.02
Pyridoxine hydrochloride	1.68	2
Riboflavin	0.32	0.219
Thiamine hydrochloride	2	2.17
Vitamin B12	0.56	0.68
i-Inositol	3.08	12.6
Inorganic Salts		
Calcium Chloride (CaCl2) (anhyd.)	93.32	116.6
Cupric sulfate (CuSO4–5H2O)	0.001	0.0013
Ferric sulfate (FeSO4–7H2O)	0.3336	0.417
Ferric Nitrate (Fe(NO3)3″9H2O)	0.04	0.05
Magnesium Chloride (anhydrous)		28.64
Magnesium Sulfate (MgSO4) (anhyd.)	68.916	48.84
Potassium Chloride (KCl)	274	311.8
Potassium Phosphate monobasic (KH2PO4)	83.2	
Sodium Bicarbonate (NaHCO3)	1960	1743
Sodium Chloride (NaCl)	5520	6995.5
Sodium Phosphate monobasic (NaH2PO4-H2O)		62.5
Sodium Phosphate dibasic (Na2HPO4) anhydrous	61.48	71.02
Zinc sulfate (ZnSO4–7H2O)	0.012	0.432
Other		
D-Glucose (Dextrose)	2240	3151
Hypoxanthine Na	1.88	2.39
Lipoic Acid	0.08	0.105
Linoleic Acid		0.042
Phenol Red	6.48	8.1
Sodium Pyruvate	88	55
Putrescine 2HCl		0.081
Thymidine	0.28	0.365
HEPES		3574.5
Ascorbic acid-2-phosphate		64
Sodium selenite		0.28
Insulin		19.4
Transferrin		10.7
FGF-2		0.1
TGF-b		0.002
Fetal Bovine Serum	(20%)	

* The values for PCGM component concentrations are 80% of those in the conventional DMEM/F10 formulation and do not include the contribution from the 20% FBS in the medium.

** The E8 formulation is essentially a modification of conventional DMEM/F12.

volumes were also recorded to allow for normalization of later analyses to the potential degrees of evaporation that took place during culture. However, the collected volumes were all within a few microliters of the 3 mL starting volumes, so normalization was not ultimately performed for the analysis. These media volumes were then filtered through 0.20 μm membrane filters into sterile tubes and stored at −80 °C for later analysis.

2.3. Glucose and lactate analysis

To measure carbohydrates and organic acids in the spent media samples, an HP 1100 high performance liquid chromatography (HPLC) system (Agilent Technologies, Santa Clara, CA) coupled with a refractive index (RI) detector (model # 1755, Bio-Rad Laboratories, Hercules, CA) was used with an Aminex HPX-87H column (Bio-Rad Laboratories). The mobile phase consisted of 5 mM sulfuric acid. Briefly, the method utilized an isocratic 0.6 mL/min flow rate for 25 min per injection, with the column kept at 50 °C. Standards of glucose and lactic acid were obtained from Sigma-Aldrich (St. Louis, MO) and dilutions were prepared fresh in mobile phase prior to analysis.

2.4. Amino acid analysis

Amino acid concentrations in fresh and spent media were measured using the REBEL analyzer (908 Devices, Boston, MA). The samples were prepared using a 0.20 μm sterile filter to remove cell debris, then diluted using REBEL diluent. Each sample was analyzed in triplicate via microfluidic capillary electrophoresis paired with high-pressure mass spectrometry (CE-HPMS). Analytes were automatically identified based on migration time and mass. Quantitation was achieved using calibration curves that were automatically generated from triplicate analysis of amino acid and vitamin standards.

2.5. Growth factor analysis

To measure the concentration of FGF2 in the spent media samples, an enzyme-linked immunosorbent assay (ELISA) kit for human FGF2 was used according to the manufacturer's instructions (ThermoFisher cat. # KHG0021). The E8 spent media samples were diluted 50-fold in sample diluent buffer while the PCGM samples were used undiluted in the assay. The final colorimetric absorbances in the 96-well assay plate were read at 450 nm using a SpectraMax iD3 multi-mode plate reader (Molecular Devices, San Jose, CA).

2.6. Calculation of yields of biomass on amino acids and on glucose

To calculate the amount of amino acid consumed to create 1 kg of wet cell mass, we focused on only the amino acids that decreased over time in either of the two media types. We first divided the mass of amino acids consumed per well from D0 to D4 by the number of new cells that were generated per well over that same time. We used an assumption of 3000 pg wet mass per cell (Alberts et al., 2017) to convert this ratio to g of AA consumed per kg of wet cell mass produced. We used the same approach to calculate biomass yields on glucose use.

3. Results

3.1. Cell growth was largely similar between the two media but slightly higher in Essential 8

By recording cell counts per well every 24 h during the culture period, we determined that the serum free E8 medium supported a similar degree of cell growth compared to the conventional serum-containing medium that we used. Fig. 1A includes representative micrographs of the two cell cultures at days 1 and 5 after plating, and shows their appearance before and after reaching confluence. Fig. 1B is a graph of the cell count data obtained by nuclear stain image analysis, and shows that the cell counts between the two media were the same through the first two days of culture. The E8 cell count increased exponentially to day 3 and more quickly reached its maximum (which was higher than any cell count in the PCGM media) but then started to decline slightly and then dropped off significantly after day 6 (likely due to increased cell death and detachment). The PCGM cell count finished exponential growth after day 2 but continued gradually increasing to day 7, only reaching the E8 cell count by day 6.

3.2. Glucose utilization and lactate accumulation were similar between the two media

We next quantified the glucose and lactate concentrations in the spent media samples that were collected on each day of the cell culture period. Fig. 2A compares the glucose utilization curves for both media types, while 2B overlays the lactate accumulation curves. The data indicate that glucose was used very similarly between the two media groups until around day 4, when the utilization slowed down for PCGM but continued for E8, despite continued cell growth in the PCGM group and decreasing cell number in the E8 group. While the initial lactate concentration in the PCGM was significantly higher than in the E8,

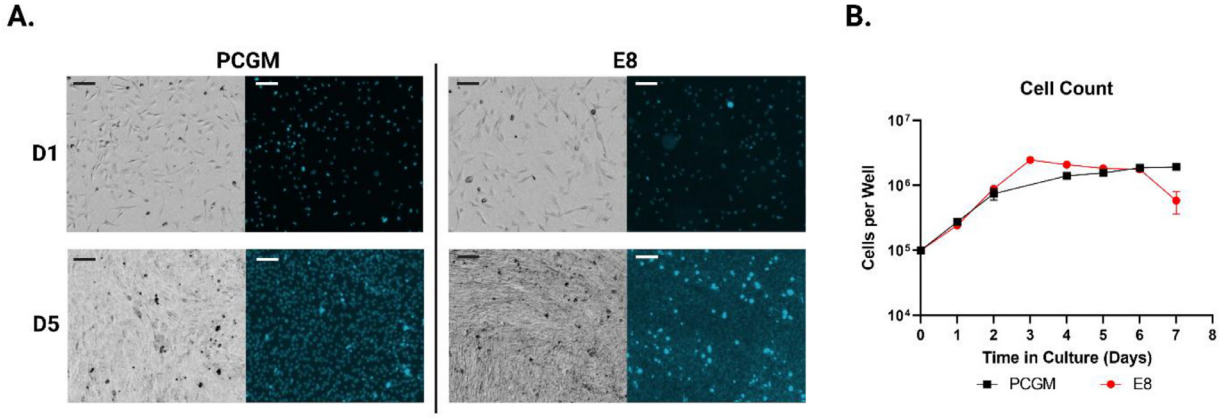

Fig. 1. Comparison of the growth characteristics of C2C12 in serum and serum-free media. A, micrographs of live cells *in situ* captured at days 1 and 5 of culture for comparison of the morphology and degree of growth of the C2C12 cells in PCGM media (20% fetal bovine serum) and E8 media (serum-free). The brightfield image on the left is paired with its corresponding DAPI channel image on the right. Cell nuclei visible in the DAPI channel were stained with Hoechst 33,342 and used to facilitate cell counting. Scale bars are 130 μm. B, cell count data of the same cells over 7 days, obtained by software image analysis using nuclear staining. Data points indicate the mean cell count of three biological replicate wells ± biological standard deviation.

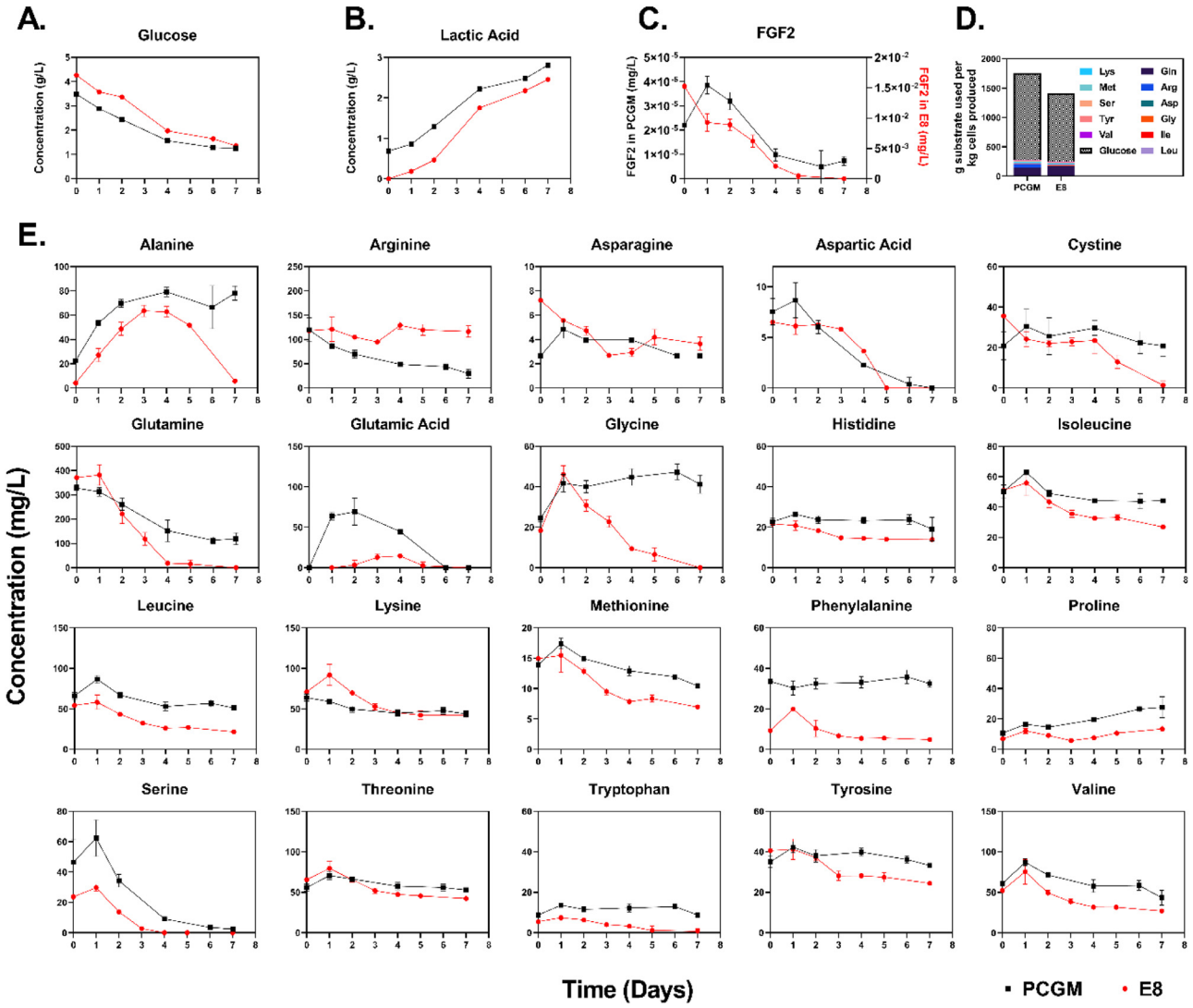

Fig. 2. Comparison of the metabolite concentrations in spent media samples over time. Samples of spent media were collected at each day of C2C12 cell culture from the serum-containing PCGM group as well as the serum-free E8 group for chemical analysis. **A**, glucose concentration curve from HPLC analysis. **B**, lactic acid concentration curve from HPLC analysis. **C**, concentration curves of basic fibroblast growth factor (FGF2) from ELISA analysis, with the curve for each media overlaid on different linear y-axis scales. **D**, analysis of cumulative substrate consumption from D0 to D4 to produce 1 kg of wet cell mass (based on an assumption of a single-cell wet mass of 3000 pg). **E**, concentration curves of free amino acids from CE-HPMS-based REBEL analysis. Note that the y-axis scales are not all the same. The data points represent mean concentration values from three biological replicate wells ± biological standard deviation.

the relative rates of lactate accumulation in the two media were similar over the seven-day culture period.

3.3. Basic fibroblast growth factor analysis

We measured the concentrations of FGF2 in our spent media samples to identify differences in utilization rates between the two media regimes, and determine whether there may be a correlation between a decline in the growth factor concentration and any particular cell growth behaviors observed during the culture period. The primary consideration to note is that the E8 media contained several orders of magnitude more FGF2 than the PCGM at the early time points of culture; this reflects their respective formulations, with the extra FGF2 in E8 used as a primary ingredient intended to replace the function of FBS.

According to the data shown in Fig. 2C, the FGF2 concentrations in both media began displaying significant reductions by around 3 or 4 days in culture, which is roughly the time the respective cell counts stopped increasing exponentially. While the FGF2 concentrations reached their minimum measurable values by around 5 or 6 days in culture for both media types, the cell numbers were maintained for at least a few additional days thereafter. Overall, there was not a significant difference in the relative FGF2 depletion patterns between the two media types.

3.4. Several key amino acids were utilized differently

According to our amino acid analysis in Fig. 2E, the majority of initial (day 0) concentrations of each amino acid were similar between PCGM and E8, congruent to their basal media formulations. Discrepancies are likely due to the unknown amino acid profile of the FBS component in PCGM. Many of the amino acid concentrations did not display a significant decrease over time for either of the media, suggesting that they are not used or are metabolically cycled by the cells. For the amino acids that were depleted, the E8 media group seemed to decrease amino acids more rapidly and/or completely compared to the cells in PCGM. For example, glutamine and glycine were significantly more depleted in the E8 at 4–5 days in culture compared to their concentrations in PCGM. Serine was depleted almost completely in E8 by around day 3 which was

the time at which exponential growth stopped. Otherwise, most amino acids showed similar general trends of utilization, except for a few, such as arginine.

3.5. Comparison of biomass yields on substrate utilization

Our data allowed us to calculate yield coefficients of biomass on amino acids and biomass on glucose. These numbers are critical for an effective technoeconomic analysis of a process like that of CM production. Fig. 2D illustrates an extrapolation from our SMA, suggesting that 252.3 g of total amino acids (including 177.7 g of glutamine) and 1157.2 g of glucose might be required to produce a kilogram of C2C12 cells when cultured in E8. Conversely, 278.1 g of total amino acids (including 143.3 g of glutamine) and 1473.6 g of glucose may be required to produce a kilogram of cells cultured in PCGM. It should be noted that some of this glucose may be directed toward metabolic byproducts like lactic acid and not solely toward biomass.

4. Discussion

Important advances in serum-free cultivated meat media development are emerging in both academia and industry and studies investigating the most effective ways to minimize and replace expensive and animal-derived components of conventional media formulations have started to be published in recent years (Andreassen et al., 2020; Karnieli et al., 2017; Kolkmann et al., 2020; Stout et al., 2022). Because it was one of the best performing media tested with bovine myoblasts by Kolkmann et al. (2020), Essential 8^{TM} has emerged as a model commercially available serum-free media for CM researchers, despite originally being developed to culture human pluripotent stem cells for biomedical research applications (Chen et al., 2011). While E8 itself did not appear to yield bovine cell growth rates comparable to serum-containing media in these previous studies, our results indicate that the C2C12 myoblast cell line does perform at least as well in E8 (Fig. 1). C2C12 is an immortalized muscle cell line that differs in important physiological ways from adult stem cells that may be more relevant to CM. However, in order to reach better process efficiency, the CM industry may need to develop their own immortalized cell lines like C2C12. Our results nevertheless reinforce the fact that significant differences exist between the media requirements of CM-relevant cells from different species and lineages, and highlight the need to optimize media specifically for each cell type.

A recent study by Jang et al. (2022) characterized the metabolite profiles of proliferating and differentiated C2C12s growing in two commercially available SFM as well as a serum-containing medium. While the two mostly proprietary SFM formulations they compared contained complex additional proteins such as albumin (unlike the E8 media we tested), they found that the differentiation state of the cells had a more profound effect on the cell metabolic activity than the type of media that was used. Our more comprehensive SMA of similar cell culture paradigms complements these findings by elucidating how the presence or absence of serum affects the specific cellular nutrient requirements.

Our examination of glucose and lactate concentration kinetics (Fig. 2A and B) suggest interesting influences of the media type on the metabolic behavior of the cells. The glucose utilization and lactate accumulation were largely similar between the serum- and serum-free media we tested, indicating the metabolic activity of the cells was not significantly different in the different media and suggesting that the absence of serum may not directly affect glucose metabolism. However, it is known that complex interactions of some of the many additional growth factors, cytokines, and other signaling molecules present in serum can regulate glycolysis and other metabolic activities (Heiden et al., 2009); these may be worth further investigation in the context of CM bioprocess optimization (Courtnay et al., 2015; Heiden et al., 2009).

The initial presence of lactate in the PCGM, coming from the FBS, is one significant variable to consider when comparing serum- versus serum-free cell culture. Chronic lactate overload has been shown to inhibit myogenic activity (Oh et al., 2019), and it will thus be important to interpret previous myoblast physiology studies that used FBS-containing media with this consideration. The substantially higher lactate concentrations in our serum-containing culture may have had a subtle role in cell proliferation inhibition, and the lower lactate concentrations that we observed in E8 media may be another benefit of using serum-free media for CM production.

The analysis of cumulative substrate input required to produce a kilogram of cells (Fig. 2D) reveals that there are not particularly large differences in glutamine, glucose, or total amino acid requirements between the two types of media. While the cells grown in PCGM appeared to require more of these substrates overall, the calculated requirements between the two media were still within 20% of each other. The actual average mass per cell at day 4 between the two media was likely different (although we were unable to directly measure that in this study and assumed a cell mass of 3000 pg in both media), and this could explain the differences in our calculated substrate requirements per kg of cells. The differences could also reflect the influence of the media formulations on the cellular metabolic activities.

Regardless, our finding that roughly 250–275 g of total amino acid would be required to produce 1 kg (wet weight) of C2C12 cells corresponds with estimates used in previous technoeconomic assessments regarding the amino acid requirement for CM cells (Humbird, 2021). Those estimates were derived from data on unrelated cell types because the specific metabolic requirements for CM-relevant cell types had not yet been evaluated. Similarly, our calculation of roughly 1100–1500 g of glucose needed to generate a kilogram of cells is congruent with these previous estimates. However, it should be noted that there is potentially a great possibility of improving biomass yields on glucose utilization. Our culture system was essentially unoptimized for efficient bioproduction. For example, many industrial bioprocess feed strategies employ the use of lower-nutrient media during the initial culture periods to help mitigate lactate production and accumulation, before introducing more glucose-rich feeds to support greater cell growth (Xie and Wang, 2006). Given that we observed substantial lactate production relatively early on, this is an indication that there is room for improvement (through cell line, media formulation, and/or feed strategy optimization) to shunt glucose metabolism to a process that produces less lactate and is more efficient for biomass production, requiring less glucose overall (Freund and Croughan, 2018; Heiden et al., 2009; Xie and Wang, 1994).

The amino acid kinetics analysis (Fig. 2E) suggests that many amino acids are not significantly depleted by cells growing in either serum-containing or serum-free media and so are potentially not essential to include in optimized CM media, or at least in such substantial quantities. However, the analysis also reveals intriguing differences between the amino acid metabolism of cells growing in the two types of media. The difference in the rates of glutamine utilization suggest that cells in E8 media were undergoing higher rates of glutaminolysis until glutamine completely ran out by around day 4; this idea is supported by the low depletion rates of aspartate and alanine (metabolites of glutaminolysis) in E8 until day 4. However, the substantially higher glutamate concentration observed in PCGM through day 4 could suggest that glutaminolysis in the PCGM cells was occurring with a lesser degree of final deamination of glutamate to α-ketoglutarate. While these types of biochemical differences in amino acid metabolism between the serum- vs serum-free media are interesting and worth exploring further, the intention of this study was simply to highlight overall tendencies in amino acid consumption rates and determine how the media formulations themselves may influence the nutrient requirements of C2C12 cells.

While the overall FGF2 utilization trends did not appear significantly different between the two media, the data in Fig. 2C, compared with the growth curve data in 1B, suggest that around 700x more cells were generated in PCGM per mg of FGF2 utilized. FGF2 was utilized more by the cells growing in the serum-free E8 media compared to PCGM, which could be due to the lack of other growth-promoting factors normally

found in serum, making the FGF2 even more important as a "substrate" for growth in SFM than it would otherwise be. The greater FGF2 in the E8 media could also explain the higher total cell number achieved in E8. FGF2 directly inhibits myoblast differentiation and promotes proliferation by altering the activity of the myogenic regulatory factor myogenin and preventing differentiation (Li et al., 1992). In this way, FGF2 could decrease contact inhibition, permitting more proliferation even as cell density increases. However, whether this degree of growth warrants the high levels of an expensive growth factor in CM media remains an open question.

Our results here provide important confirmation of general assumptions on media substrate requirements and yield expectations for CM production that will be highly useful for further technoeconomic analyses. Relatively little work has previously been completed on characterizing the metabolic requirements of the most CM-relevant cell types, so many of the more specific metabolic assumptions being made about them are derived from knowledge of how these cell types behaved in earlier studies using serum-containing media for other scientific purposes. Our study indicates that these assumptions may be disadvantageous to the overall ease and success of transitioning the cells to completely serum-free and chemically-defined media. A significant amount of time and effort will potentially be needed—utilizing SMA, black-box machine learning, and other biological insights—to properly optimize serum-free media for these cell types to achieve the most efficient CM bioprocesses at lowest cost (Cosenza et al., 2021, 2022). Perhaps, cell lines for CM could be created to be more robust to the effects of various SFM formulations to allow for greater versatility, and this may be a worthwhile pursuit for academic CM researchers in the future.

Ethical statement

The authors declare that this manuscript did not involve human or animal studies and did not require special ethical consideration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Edward N. O'Neill: Conceptualization, Methodology, Writing – original draft, Formal analysis, Data curation, Visualization, Writing – review & editing. **Milla Neffling:** Formal analysis, Writing – original draft, Writing – review & editing. **Nick Randall:** Formal analysis, Writing – original draft, Writing – review & editing. **Grace Kwong:** Writing – original draft, Writing – review & editing. **Joshua Ansel:** Writing – original draft, Writing – review & editing. **Keith Baar:** Conceptualization, Methodology, Data curation, Funding acquisition, Supervision, Writing – review & editing. **David E. Block:** Conceptualization, Methodology, Data curation, Funding acquisition, Supervision, Writing – review & editing.

Data availability

Data will be made available on request.

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