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Metabolic rate does not scale with body mass in cultured mammalian cells

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Brown MF, Gratton TP, Stuart JA. Metabolic rate does not scale with body mass in cultured mammalian cells. *Am J Physiol Regul Integr Comp Physiol* 292: R2115–R2121, 2007. First published January 18, 2007; doi:10.1152/ajpregu.00568.2006.—The allometric scaling of metabolic rate with organism body mass can be partially accounted for by differences in cellular metabolic rates. For example, hepatocytes isolated from horses consume almost 10-fold less oxygen per unit time as mouse hepatocytes [Porter and Brand, *Am J Physiol Regul Integr Comp Physiol* 269: R226–R228, 1995]. This could reflect a genetically programmed, species-specific, intrinsic metabolic rate set point, or simply the adaptation of individual cells to their particular in situ environment (i.e., within the organism). We studied cultured cell lines derived from 10 mammalian species with donor body masses ranging from 5 to 600,000 g to determine whether cells propagated in an identical environment (media) exhibited metabolic rate scaling. Neither metabolic rate nor the maximal activities of key enzymes of oxidative or anaerobic metabolism scaled significantly with donor body mass in cultured cells, indicating the absence of intrinsic, species-specific, cellular metabolic rate set points. Furthermore, we suggest that changes in the metabolic rates of isolated cells probably occur within 24 h and involve a reduction of cellular metabolism toward values observed in lower metabolic rate organisms. The rate of oxygen delivery has been proposed to limit cellular metabolic rates in larger organisms. To examine the effect of oxygen on steady-state cellular respiration rates, we grew cells under a variety of physiologically relevant oxygen regimens. Long-term exposure to higher medium oxygen levels increased respiration rates of all cells, consistent with the hypothesis that higher rates of oxygen delivery in smaller mammals might increase cellular metabolic rates.

allometry; oxygen tension; supply networks; metabolic regulation; fibroblast; respiration

RUBNER (18) and Kleiber (10) first introduced the observation that mammalian basal metabolic rate per unit mass scales inversely with body mass. Thus, larger mammalian species expend less energy per unit mass to maintain their soma than do smaller species. These differences are explained at least partially by differences in cellular metabolic rates between species. Kleiber (11) determined that the metabolic rate per unit weight of liver tissue is inversely proportional to body mass of the host animal. Krebs (13) extended this observation to other tissues, demonstrating similar relationships for tissue slices from brain, kidney, liver, lung, and spleen. Porter and Brand (17), demonstrated the persistence of metabolic rate scaling in a homogeneous population of freshly isolated cells: horse hepatocytes consume almost 10-fold less oxygen per unit time than those from mice. Similarly, Singer et al. (20) demonstrated allometric scaling of metabolic output in mixed populations of mammalian blood cells.

Do the higher metabolic rates of the cells of smaller species reflect intrinsic metabolic properties or do they arise secondarily as an adaptation of the cell to its environment, i.e., conditions within the host animal? Wheatley and Clegg (26) suggested that the existence of intrinsic metabolic rate set points could be examined in a cell culture environment, where the medium in which cells of different species are bathed is standardized and therefore does not contribute to between-species differences. Theoretical analyses of metabolic rate scaling predict that cells growing in vitro should adopt identical metabolic rates (25). However, original data validating this hypothesis are lacking and a thorough study, in a single laboratory using the same cell type from a range of mammalian species growing in culture under a well-defined set of conditions is required. Testing the hypothesis that metabolic rate scaling will be absent in cultured cells requires measurements of metabolic parameters in stable populations of cells capable of being propagated in vitro for several generations. Here we have used primary dermal fibroblasts selected from 10 mammalian species, with donors ranging in body mass over five orders of magnitude from 5 to 600,000 g, to test the hypothesis that metabolic rate scaling is absent in cells growing in culture (26, 25).

Some authors (4, 5, 23–25) have suggested that the lower cellular metabolic rates of larger mammals represent an adaptation to nutrient supply limitation that is inherent to mammalian cardiovascular design. Specifically, larger mammals may not be able to supply oxygen to somatic cells at sufficiently high rates to support cellular metabolic rates similar to those of mice (4, 5). We have examined the idea that oxygen supply could be a contributing factor in the scaling of cellular metabolic rates by maintaining cells in culture under different extracellular oxygen regimens, then measuring the resultant steady-state rates of oxygen consumption under identical conditions (atmosphere-saturated media).

MATERIALS AND METHODS

Materials. Modified Eagle's medium with Earl salts, L-glutamine, and sodium bicarbonate were obtained from Sigma-Aldrich (St. Louis, MO). Penicillin/streptomycin, nonessential amino acids and fetal bovine serum was obtained from Hyclone (Logan, UT). All other chemicals and purified enzymes were obtained either from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Fair Lawn, NJ), unless otherwise stated.

Cell lines and growth conditions. Rat (*Rattus norvegicus*) and rabbit (*Oryctolagus cuniculus*) primary dermal fibroblast cells were acquired from the American Type Culture Collection (Manassas, VA). Sheep (*Ovis aries*), cat (*Felis catus*), dog (*Canis familiaris*), cow (*Bos taurus*), monkey (*Macaca fascicularis*), horse (*Equus caballus*), and human (*Homo sapiens*) primary dermal fibroblast cells were

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Table 1. Primary dermal fibroblasts used in this investigation

Repository No.	Species	Body Mass, g	Age at Biopsy	Sex	PDL Range
CRL-1213	<i>Rattus norvegicus</i>	5	18 Days gestation	Unknown	5–9
None	<i>Mus musculus</i>	35	Adult	Female/Male	3–5
GM06207	<i>Felis catus</i>	3,000	Adult	Male	6–10
CRL-1414	<i>Oryctolagus cuniculus</i>	4,000	Adult	Female	8–11
AG07388	<i>Canis familiaris</i>	6,000	Adult	Female	3–6
GM03446	<i>Macaca fascicularis</i>	7,000	Unknown	Male	7–11
GM03550	<i>Ovis aries</i>	20,000	4 mo	Female	4–8
GM00037	<i>Homo sapiens</i>	57,000	Adult	Female	16–17
AG07906	<i>Equus caballus</i>	450,000	Adult	Female	4–6
GM06034	<i>Bos taurus</i>	600,000	Adult	Male	11–15

PDL, population-doubling level.

acquired from the Coriell Institute. Mouse (*Mus musculus*) dermal fibroblasts were provided by Richard A. Miller and Adam Salmon (University of Michigan, Ann Arbor, MI). Repository number, age at biopsy, body mass, and population-doubling level of each species are outlined in Table 1. All cell lines were cultured under identical conditions [37°C, humidified to 5% CO₂-18% O₂ atmosphere in modified Eagle's medium (with Earl salts) with L-glutamine and sodium bicarbonate, and supplemented with 100 U/ml penicillin/streptomycin, nonessential amino acids, and 15% fetal bovine serum], with the exception that mouse fibroblasts were cultured in Dulbecco's modified Eagle's medium, 10% fetal bovine serum, and 0.25 µg/ml Fungizone. Cell lines were also cultured under low-oxygen conditions: 5% CO₂-5% O₂, 5% CO₂-3% O₂, and 5% CO₂-1% O₂.

Oxygen consumption measurements. Cellular oxygen consumption was measured at 37°C in triplicate 2-ml aliquots of cell suspension by using a Rank Brothers Dual digital (model 20) respirometer fitted with a water-jacketed cell chamber. Cell sheets were harvested from two to five 100-mm plates by trypsinization. Harvested cells were washed twice in PBS. Cell viability was determined by the exclusion of 0.4% (wt/vol) trypan blue and in all preparations viability was >95%. Approximately 200 µl of cell suspension (10⁶ cells) was added to the respirometry chamber. Respiration rates were determined once a stable linear rate was obtained. To determine nonmitochondrial oxygen consumption rates, 3 mM KCN was added to the cell suspension and the respiration rate was determined again.

Preparation of whole cell extracts. Whole cell extracts were prepared from four to eight 100-mm plates of each cell line. Cells were scraped from dishes into 50-ml conical tubes, centrifuged at 200 g for 3 min and washed twice with PBS. Cells were resuspended and incubated for 1 h with periodic sonication (Ultrasonic Sonicator W-375) in 0.5 ml of ice-cold lysis buffer (10 mM Tris pH 8.0, 150 mM NaCl, 2 mM EDTA, 2 mM DTT, 0.4 mM PMSF, 40% glycerol, and 0.5% Nonidet P-40). Following incubation, cell lysates were centrifuged at 16,000 g, 4°C for 10 min (accuSpin Micro R; Fisher

Table 2. O₂ consumption in primary dermal fibroblast of mammals of varying size

Species	Body Mass	Total O ₂ Consumption	Mitochondrial-Dependent O ₂
<i>R. norvegicus</i>	5	5.14 ± 0.53	3.76 ± 0.70
<i>O. cuniculus</i>	4,000	2.35 ± 0.25	2.12 ± 0.23
<i>C. familiaris</i>	6,000	3.68 ± 0.70	3.08 ± 0.50
<i>M. fascicularis</i>	7,000	2.54 ± 0.38	2.03 ± 0.36
<i>O. aries</i>	20,000	1.93 ± 0.16	1.83 ± 0.16
<i>H. sapiens</i>	57,000	3.64 ± 0.02	2.78
<i>E. caballus</i>	450,000	2.53 ± 0.26	2.22 ± 0.96
<i>B. taurus</i>	600,000	4.69 ± 0.24	3.26 ± 0.60

Each value represents the mean ± SE of 3 independent measurements (except mitochondrial-dependent oxygen consumption of *H. sapiens* and *E. caballus*, which represent 1 and 2 independent measurements, respectively). Body mass values are in grams; O₂ values = nmol·min⁻¹·10⁶ cells⁻¹.

Scientific). Protein content of the cell lysates was determined by the Bradford method using a Bio-Rad protein assay kit. Lysates were stored at -80°C.

Enzyme assays. Enzyme activities were determined using a Varian Cary 100 Bio UV-Visible Spectrophotometer equipped with a Peltier

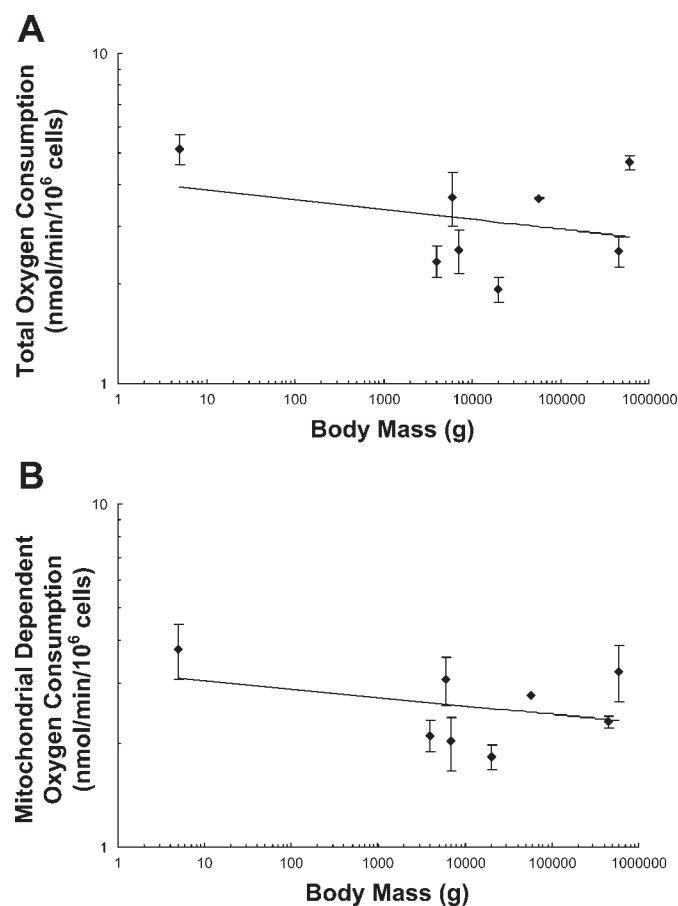


Fig. 1. Dermal fibroblast O₂ consumption as a function of body mass. A: log-log plot showing the lack of relationship between dermal fibroblast total O₂ consumption rate and species body mass ($F_{1,6} = 0.630$, $P = 0.457$). B: log-log plot of mitochondrial-dependent O₂ consumption rate as a function of body mass. Mitochondrial-independent O₂ consumption was determined by inhibition with 3 mM KCN and subtracted from the overall rate to determine the mitochondrial-dependent rate. No relationship was found between mitochondrial-dependent O₂ consumption and body mass ($F_{1,6} = 0.796$, $P = 0.407$). Data points represent the means ± SE of three independent measurements with the exception of mitochondrial-independent O₂ consumption measurements for human and horse are one and two independent measurements, respectively.

Table 3. Statistical analysis of linear regressions, enzyme activity as a function of body mass

Metabolic Activity	Correlation Coefficient	Slope	F Value	P Value
Total oxygen consumption	0.095	-0.030	0.630	0.457
Mitochondrial-dependent oxygen consumption	0.131	-0.026	0.796	0.407
Citrate synthase @ 18% O ₂	0.010	-0.011	0.081	0.783
Citrate synthase @ 3% O ₂	0.217	-0.070	1.944	0.206
Cytochrome <i>c</i> oxidase	0.028	0.034	0.231	0.644
Lactate dehydrogenase @ 18% O ₂	0.073	-0.030	0.629	0.450
Lactate dehydrogenase @ 3% O ₂	0.104	-0.047	0.814	0.397
Pyruvate kinase	0.002	-0.008	0.018	0.898

thermostatable cell changer maintained at 30°C. Citrate synthase was monitored at 412 nm. The assay buffer contained 50 mM Tris pH 8.0, 0.5 mM 5,5'-dithiobis(2-nitrobenzoic acid), 0.1 mM acetyl-coenzyme A, 0.05% Triton X-100 and 10 µg protein. The reaction was initiated by the addition of 0.5 mM oxaloacetate, and absorbance was followed for 7 min. Cytochrome *c* oxidase was monitored at 550 nm by using fully reduced cytochrome *c*. Cytochrome *c* was fully reduced with the addition of sodium dithionite and passed through a Sephadex G25 column to remove excess dithionite. The assay buffer contained 25 mM potassium phosphate buffer pH 7.2, 0.5% Tween 20 and 10 µg protein. The reaction was initiated by the addition of 50 µM fully reduced cytochrome *c* and absorbance was followed for 3 min. Pyruvate kinase and lactate dehydrogenase reaction rates were determined by the increase or decrease in the absorbance of NADH or NADPH at 340 nm. Pyruvate kinase assay buffer contained 20 mM HEPES pH 7.4, 0.2 mM NADH, 5 mM ADP, 50 mM KCl, 10 mM MgCl₂, 5 units lactate dehydrogenase, and 5 µg protein. The reaction was initiated by the addition of 5 mM phosphoenolpyruvate, and absorbance was followed for 3 min. Lactate dehydrogenase assay buffer contained 20 mM HEPES pH 7.3, 0.2 mM NADH and 2 µg protein. The reaction was initiated by the addition of 10 mM pyruvate, and absorbance was followed for 3 min.

Statistical analyses. Regression ANOVA analysis was carried out on oxygen-consumption rates and all maximal metabolic rates to determine whether the slopes of the line of best fit was significantly different from zero. Student's *t*-test analysis was used to determine the statistical significance of the differences between means of total oxygen consumption of cells grown at high vs. low oxygen concentrations. A Student's *t*-test was further used to compare the linear regression equations of citrate synthase and lactate dehydrogenase activities of cells grown at 18% and 3% oxygen.

RESULTS

Metabolic rates of dermal fibroblasts maintained in culture do not scale to body mass. To determine whether the allometric scaling relationship between cellular metabolic rate and body mass holds for cells propagated in culture, oxygen consumption rates of dermal fibroblasts from mammal species ranging from 5 to 600,000 g (covering 5 orders of magnitude) were

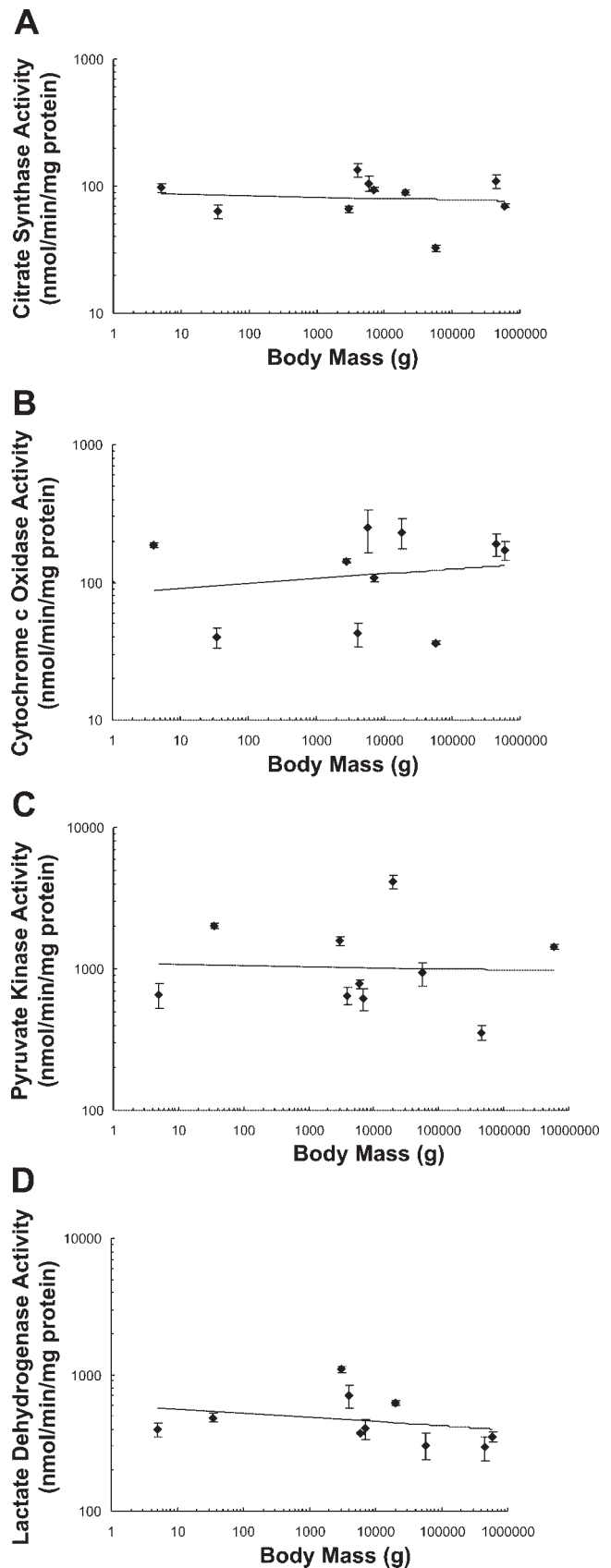


Fig. 2. Lack of a relationship between metabolic enzyme activities of cultured dermal fibroblasts and species body mass. Log-log plots of citrate synthase activity (A) and cytochrome *c* oxidase activity (B) as a function of body mass. Neither correlation was statistically significant: citrate synthase ($F_{1,8} = 0.081$, $P = 0.783$) and cytochrome *c* oxidase ($F_{1,8} = 0.231$, $P = 0.644$). Log-log plot of lactate dehydrogenase activity (C) and pyruvate kinase activity (D) as a function of body mass. Neither correlation was statistically significant: lactate dehydrogenase ($F_{1,8} = 0.629$, $P = 0.450$) and pyruvate kinase ($F_{1,8} = 0.018$, $P = 0.898$). Data represent the means \pm SE of triplicate measurements of 3 independently prepared cell lysates.

measured. While respiration rates ranged from 1.93 ± 0.16 to $5.14 \pm 0.53 \text{ nmol} \cdot \text{min}^{-1} \cdot 10^6 \text{ cells}^{-1}$ (Table 2), no correlation was observed between cellular metabolic rate and body mass of the host species ($P = 0.457$) (Fig. 1A; Table 3). To ensure that our oxygen consumption measurements were indicative of mitochondrial respiration, the respiratory poison potassium cyanide (3 mM) was then added to the respirometer. By this method, greater than 75% of cellular oxygen consumption was determined to be mitochondrial in all cells (Table 2). Mitochondrial-dependent cellular oxygen consumption also showed no significant scaling relationship with species body mass ($P = 0.407$) (Fig. 1B; Table 3). Thus, we found no evidence for allometric scaling of metabolic rate with species body mass in dermal fibroblasts over five orders of magnitude. The analysis was repeated after excluding the influence of the fetal rat cell data point, but, again, no significant scaling relationship was found.

The effect of cell passage number in culture on metabolic rate was also investigated. No significant difference in metabolic rate between earlier (passage 3) $2.03 \pm 0.13 \text{ nmol} \cdot \text{min}^{-1} \cdot 10^6 \text{ cells}^{-1}$ and later (passage 7) $2.33 \pm 0.41 \text{ nmol} \cdot \text{min}^{-1} \cdot 10^6 \text{ cells}^{-1}$ passaged horse cells was found. Therefore, the number of passages in culture was apparently not affecting cellular metabolic rate, at least at these latter passages.

Maximal metabolic enzyme activities of dermal fibroblasts maintained in culture do not scale to body mass. Activities of enzymes of oxidative metabolism scale inversely with body mass (6). We investigated whether any relationship exists between species body mass and activities of the mitochondrial enzymes citrate synthase and cytochrome *c* oxidase. Citrate synthase activities ranged from $32.4 \pm 1.7 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg}$ lysate protein⁻¹ (human) to $134.2 \pm 15.6 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg}$ lysate protein⁻¹ (rabbit), but showed no relationship with species body mass ($P = 0.783$) (Fig. 2A; Table 4). Similarly, cytochrome *c* oxidase activities ranged from $36.3 \pm 1.0 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg}$ lysate protein⁻¹ (human) to $248.6 \pm 83.0 \text{ nmol} \cdot \text{min}^{-1} \cdot \text{mg}$ lysate protein⁻¹ (dog), but showed no relationship to species body mass ($P = 0.644$) (Fig. 2B; Table 4).

Cells in culture medium rely primarily upon glucose to fuel both aerobic and anaerobic metabolism. We measured pyruvate kinase activity as a gauge of glycolytic flux. To address the possibility that cells from smaller species may support higher metabolic rates in culture with anaerobic glycolysis, we

also measured the activity of lactate dehydrogenase. However, neither of these activities showed any relationship with species body mass ($P = 0.898$ and $P = 0.450$, respectively) (Fig. 2, C and D; Table 4).

Taken together, these results indicate the absence of an intrinsic set point governing the different metabolic rates of cells from different species. Since oxygen supply has been suggested as an important determinant of the allometric scaling relationships observed in vivo (2–5, 23–25), and cells grown in culture face an identical oxygen regimen (equilibration of media with atmospheric oxygen), we sought to investigate whether changing oxygen concentration of the culture medium could affect cellular metabolic rate.

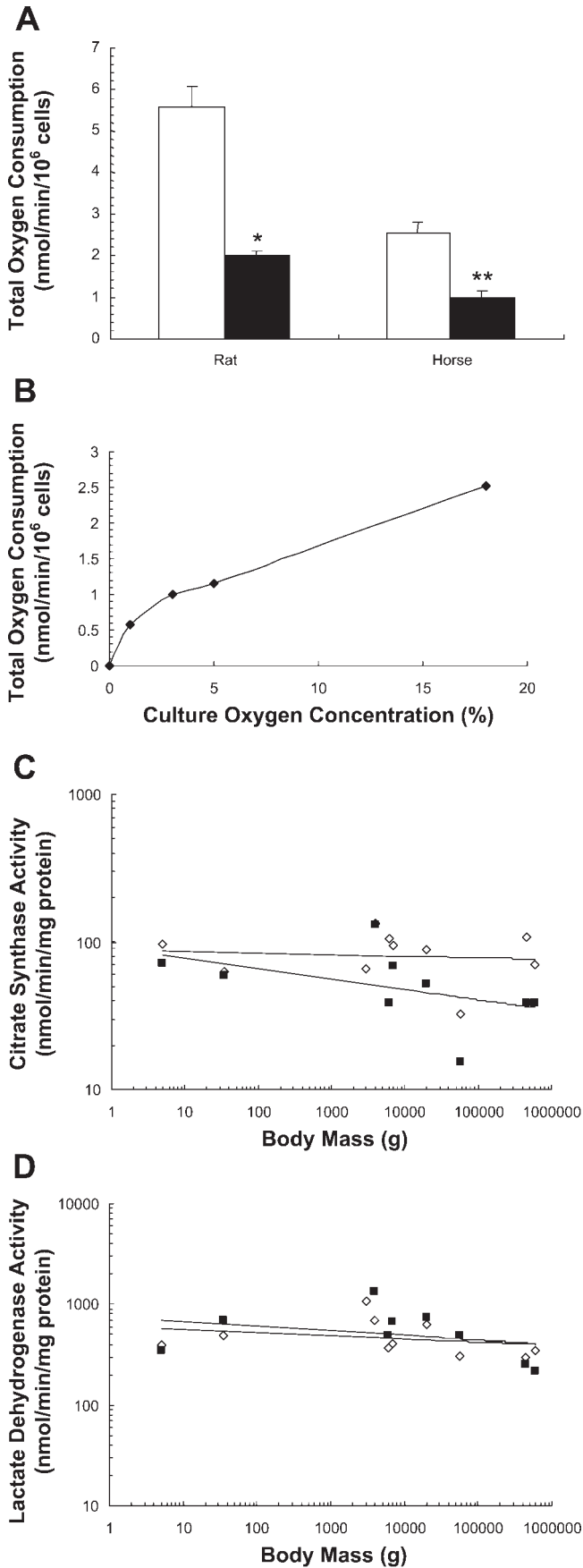
Dermal fibroblasts cultured under higher oxygen tensions have increased metabolic rates. To establish whether medium [O₂] affects metabolic rate of cells in culture, cell lines were grown for 1–2 wk at incubator oxygen concentrations of 3% and 18%, followed by measurement of respiration rates in oxygen-saturated media. Both rat and horse cells grown at 18% oxygen had significantly higher metabolic rates than those grown at 3% ($P < 0.05$ for rat and $P = 0.01$ for horse) (Fig. 3A). The concentration of dissolved oxygen in culture media was determined by removing samples of media from culture plates and measuring oxygen activity in a calibrated respirometer. Media at 18% oxygen had a dissolved oxygen concentration of $\sim 166 \text{ nmol O}_2/\text{ml}$, whereas media at 1% oxygen had a dissolved oxygen concentration of $\sim 50 \text{ nmol O}_2/\text{ml}$. This latter value is close to the physiological value for capillary blood (75 nmol O₂/ml). Horse cells were subsequently cultured at 1, 3, 5, and 18% incubator oxygen concentrations to investigate the nature of the relationship between growth medium oxygen concentration and cellular metabolic rate. Oxygen concentration had a considerable effect on cellular metabolic rates over a physiologically relevant range (Fig. 3B).

Citrate synthase activity was measured in lysates prepared from cells cultured at 3% and 18% oxygen and was typically lower (Student's *t*-test) under the former conditions (Fig. 3C; Table 4). The slopes of the regression lines for 3 and 18% conditions were, however, not statistically significant ($t = 0.67$, $t_{\text{crit}} = 2.131$), indicating that citrate synthase activity did not scale under either oxygen regimen. Furthermore, at low oxygen tensions, there was no increase in the ratio of mitochondrial dependent oxygen consumption to total (data not

Table 4. Enzyme activities of primary dermal fibroblasts from mammals spanning five orders of magnitude in size

Species	Body Mass, g	Enzyme Activity, nmol · min ⁻¹ · mg protein ⁻¹					
		Citrate Synthase		Cytochrome <i>c</i> Oxidase, 18% O ₂	Lactate Dehydrogenase		Pyruvate Kinase, 18% O ₂
		18% O ₂	3% O ₂		18% O ₂	3% O ₂	
<i>R. norvegicus</i>	5	96.8 ± 6.7	72.1 ± 4.7	186 ± 6.6	395 ± 44	349 ± 9.5	654 ± 130
<i>M. musculus</i>	35	62.8 ± 7.5	58.6 ± 6.5	39.7 ± 6.4	485 ± 36	690 ± 76	2,020 ± 95
<i>F. catus</i>	3,000	65.8 ± 3.8	N/A	143 ± 4.0	1,090 ± 65	N/A	1,580 ± 120
<i>O. cuniculus</i>	4,000	134 ± 16	131 ± 5.8	42.2 ± 8.1	701 ± 130	1,340 ± 60	649 ± 94
<i>C. familiaris</i>	6,000	105 ± 14	38.4 ± 5.6	249 ± 83	374 ± 9.2	495 ± 53	783 ± 60
<i>M. fascicularis</i>	7,000	94.2 ± 3.1	69.4 ± 13	108 ± 7.6	406 ± 70	678 ± 83	614 ± 110
<i>O. aries</i>	20,000	89.4 ± 1.9	52.5 ± 5.9	231 ± 56	622 ± 31	729 ± 32	4,160 ± 410
<i>H. sapiens</i>	57,000	32.4 ± 1.7	15.1 ± 4.9	36.3 ± 1.0	305 ± 65	494 ± 82	936 ± 170
<i>E. caballus</i>	450,000	109 ± 14	38.9 ± 5.5	190 ± 36	293 ± 58	258 ± 82	355 ± 42
<i>B. taurus</i>	600,000	70.0 ± 1.9	38.4 ± 5.5	171 ± 28	351 ± 27	217 ± 27	1,440 ± 52

Each value represents the mean ± SE of triplicate measurements of 3 lysates.



shown). Taken together, these results are consistent with a proliferation of mitochondria in cells grown at higher oxygen levels.

Lactate dehydrogenase activity was measured as an indicator of reliance on anaerobic metabolism. However, we again found no difference between the regression line describing lactate dehydrogenase activity and species body mass for cells grown at 3% ($P = 0.397$) or 18% oxygen ($P = 0.450$) (Fig. 3D; Table 4).

DISCUSSION

Various reports have suggested that the allometric scaling of cellular metabolic rates with species body mass should be absent under culture conditions, when the cells are removed from the influence of the body (25, 26). However, no original data have been provided to demonstrate this. Here we have used a standardized set of culture conditions and cell types to show that metabolic rate scaling is indeed absent from cells propagated in culture.

We used primary dermal fibroblasts, which are connective tissue cells that function in synthesizing and maintaining the extracellular matrix, participate in wound healing and can dedifferentiate to other cell types. Fibroblasts are a useful model for a study of this nature because they constitute a homogeneous cell population that can replicate and therefore be maintained over relatively long periods in vitro, and they are available from a wide range of mammalian species. Changes in the metabolic rates of these cells in a culture environment should therefore represent adaptation to in vitro culture conditions rather than simply a gradual loss of function preceding death. In contrast, cells isolated from large tissue masses (e.g., muscle, brain, liver, or kidney) can be maintained in vitro but do not readily replicate and thus have limited useful life spans in culture during which changes may be attributed to a gradual loss of viability. In addition, slices from these tissues (13) will contain heterogeneous cell populations, which may differ in proportion between species, thus limiting interpretation of the cellular basis of allometric relationships. On the other hand, in fibroblast cell lines initiated from mammalian tissues, the fibroblasts become the predominant cell type after only a few passages because of their replicative advantage.

Due to the nature of culture establishment, it is not possible to determine the metabolic rate of a homogenous population of fibroblasts immediately following biopsy. As a result, initial changes in metabolic rate could not be determined, nor could we establish whether the metabolic rates of dermal fibroblasts

Fig. 3. Respiration rates of dermal fibroblasts are higher when cells are grown at higher O₂ concentrations. A: respiration in rat and horse fibroblasts (measured in fully oxygenated medium) that were grown at 18% (white bars) and 3% (black bars) O₂ (* $P < 0.05$; ** $P = 0.01$). B: relationship between %O₂ under culture conditions and subsequent respiration rates of horse dermal fibroblasts. Cells were grown at 1, 3, 5, or 18% O₂, and respiration rates were measured in atmosphere-saturated medium in harvested cells as above. C and D: lack of correlation between species mass and metabolic enzyme activities of cells grown at 18% (white diamonds) or 3% (black squares) O₂. C: citrate synthase activity in cells grown at 3% O₂ ($F_{1,7} = 1.944$, $P = 0.206$). D: lactate dehydrogenase activities of cells grown at 3% O₂ ($F_{1,7} = 0.814$, $P = 0.397$). Data are the means \pm SE of triplicate measurements on 3 lysates.

scale in vivo. However, we did investigate whether changes in metabolic rate were occurring in early passaged horse fibroblasts. Early passaged (passage 3) horse cells did not differ significantly from later passaged cells (passage 7). Therefore, changes in metabolism may have occurred very early in culture. Limited available evidence suggests that other cell types undergo metabolic rate changes within the first 24 h following isolation. Initial (within 6 h) metabolic rates of isolated rat hepatocytes are higher than those measured after 24 h (Table 5). Primary rat hepatocytes maintained for several days in so-called "bioartificial livers" also have similarly lower rates of oxygen consumption, as do transformed hepatocytes maintained in long-term culture (Table 5). Based on these data, we suggest that ex vivo changes in metabolic rate occur within the first 24 h and are maintained in culture thereafter, unless conditions (e.g., oxygen tension) are varied.

It is interesting to ask in which direction cellular metabolic rate changes in culture, i.e., do cells from smaller animals with higher metabolic rates undergo a decline or vice versa? Rat hepatocytes have metabolic rates ranging from 24–40 $\text{nmol}\cdot\text{min}^{-1}\cdot 10^6\text{ cells}^{-1}$ when measurements are made within 10 h or less, postisolation (17, 19). But metabolic rate measurements after 24 h have been reported as between 3.8 and 5.4 $\text{nmol}\cdot\text{min}^{-1}\cdot 10^6\text{ cells}^{-1}$, rates that are apparently maintained stably for several days in vitro (15, 16). These values are similar to those reported for freshly isolated horse hepatocytes (6 $\text{nmol}\cdot\text{min}^{-1}\cdot 10^6\text{ cells}^{-1}$) (17), which suggests that cells from organisms with higher metabolic rates undergo a relatively rapid reduction of metabolism in culture to become more like their lower metabolic rate counterparts. Indeed, hepatocytes maintained in vitro have metabolic rates that are similar to those of fibroblasts and other cell types, regardless of host species (Table 5). Thus, any cells removed from the environment of the whole organism, bathed in the same nutrient medium, and no longer performing their normal in vivo functions, may adopt the same relatively low metabolic rates that are comparable to in vivo values of large mammals.

A possible explanation for the absence of metabolic rate scaling in fibroblasts is that it may only be observed in cell types that contribute significantly to the basal metabolic rate of

the organism. Many previous reports involving metabolic scaling with species body mass have studied highly metabolically active tissues/cells, such as liver, brain, and muscle. However, metabolic rates of less "active" tissues, such as lung, spleen (13), and blood cells (20) also scale negatively with body mass. We therefore think it is unlikely that dermal fibroblasts are a unique cell type in which metabolic rate does not scale in vivo.

Some authors (2–5, 23–25) have proposed a "single-cause" explanation for allometric scaling relationships. This theory suggests that the design of the mammalian cardiovascular system imposes constraints on rates of nutrient delivery, limiting the cellular metabolic rates of larger species to those that can be maintained by the delivery system. This "supply-side" explanation for allometric scaling is contentious (1, 21). However, elements of the hypothesis are testable in cell culture. We investigated whether oxygen supply could affect steady-state cellular metabolic rates by growing cells under different Po_2 regimens. We selected values similar to those reported for mammalian tissues (~ 30 – $110\text{ nmol O}_2/\text{ml}$) (8). Long-term exposure to specific oxygen regimens did indeed significantly affect the steady-state metabolic rates of fibroblasts (measured at constant Po_2), with cells grown at higher Po_2 adopting higher rates of oxygen consumption. A similar result has been reported for other cell types. Freshly isolated hepatocytes exposed to higher oxygen concentrations for periods of hours have higher metabolic rates (19). We think this may be related to oxygen's toxicity negatively influencing the efficiencies of cellular functions.

Thus, regardless of cell type (fibroblasts vs. hepatocytes) and state (freshly isolated vs. long-term culture), chronic exposure to high oxygen concentrations appears to similarly affect an increase in cellular metabolic rate under otherwise constant conditions (Table 5). This indicates that any body mass-related differences in average pericellular Po_2 present in vivo might contribute to allometric scaling of cellular metabolic rates in mammals. Limited available data suggest that intracellular Po_2 is relatively constant, at least in some tissues, regardless of animal size (7). However, we are not aware of definitive measurements published for a wide range of mammal species,

Table 5. Oxygen consumption rates of isolated, primary, and transformed cells from various mammal species

Cell Type	Condition	Timing of Measurement	Po_2 in Medium	Donor Species	O_2 Consumption	Reference No.
Hepatocyte	Isolated	<6 h	N/A	Rat	24.0	17
Hepatocyte	Isolated	<10 h	45	Rat	23.3	19
Hepatocyte	Isolated	<10 h	150	Rat	40.0	19
Hepatocyte	Isolated	0–24 h	212*	Rat	3.80	15
Hepatocyte	Isolated	<6 h	N/A	Horse	6.00	17
Hepatocyte	Isolated	<48 h	166	Pig	4.98	16
Hepatocyte	Transformed (Hep G2)	Long-term culture	212	Human	1.42	15
Hepatocyte	Transformed (Hep Z)	Long-term culture	≈ 332	Human	1.35	22
Macrophage	Transformed (RAW264.7)	Long-term culture	166	Mouse	1.92	14
Osteoblast	Primary	Long-term culture	166	Fetal rat	0.67	12
Fibroblast	Primary	Long-term culture	166**	Human	3.00	9
Fibroblast	Primary	Long-term culture	166**	Human	3.46	Present study
Fibroblast	Primary	Long-term culture	166**	Rat	5.59	Present study
Fibroblast	Primary	Long-term culture	64	Rat	2.00	Present study
Fibroblast	Primary	Long-term culture	166	Horse	2.53	Present study
Fibroblast	Primary	Long-term culture	64	Horse	1.00	Present study

Values for Po_2 in medium = $\text{nmol O}_2/\text{ml}$ and oxygen consumption = $\text{nmol}\cdot\text{min}^{-1}\cdot 10^6\text{ cells}^{-1}$. *Input Po_2 , no measurement for output; ** approximate measurement (actual measurements made on horse cells).

and such information would be interesting in light of these observations.

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