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NanoSIMS imaging reveals unexpected heterogeneity in nutrient uptake by brown adipocytes

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Abstract

Heterogeneity in the metabolic properties of adipocytes in white adipose tissue has been well documented. We sought to investigate metabolic heterogeneity in adipocytes of brown adipose tissue (BAT), focusing on heterogeneity in nutrient uptake. To explore the possibility of metabolic heterogeneity in brown adipocytes, we used nanoscale secondary ion mass spectrometry (NanoSIMS) to quantify uptake of lipids in adipocytes interscapular BAT and perivascular adipose tissue (PVAT) after an intravenous injection of triglyceride-rich lipoproteins (TRLs) containing [^{2}H]triglycerides (^{2}H-TRLs). The uptake of deuterated lipids into brown adipocytes was quantified by NanoSIMS. We also examined ^{13}C enrichment in brown adipocytes after administering [^{13}C]glucose or ^{13}C-labeled mixed fatty acids by gastric gavage. The uptake of ^{2}H-TRLs-derived lipids into brown adipocytes was heterogeneous, with ^{2}H enrichment in adjacent adipocytes varying by more than fourfold. We also observed substantial heterogeneity in ^{13}C enrichment in adjacent brown adipocytes after administering [^{13}C]glucose or [^{13}C]fatty acids by gastric gavage. The uptake of nutrients by adjacent brown adipocytes within a single depot is variable, suggesting that there is heterogeneity in the metabolic properties of brown adipocytes.

Keywords

Brown adipose tissue; White adipose tissue; NanoSIMS imaging; Nutrient uptake; Fatty acids

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1. Introduction

There is substantial heterogeneity in the gene expression, metabolism, and morphology of adipocytes in different white adipose tissue (WAT) depots [1, 2]. Even within a single WAT depot, the properties of adipocytes are heterogenous. For example, some adipocytes (beige adipocytes) are capable of responding to cAMP with high levels of UCP1 expression and high respiration rates [3]. Also, Boumelhem *et al.* [4] found heterogeneity in the expression of CD36, a fatty acid transporter, in WAT depots, and Valamov *et al.* [5] observed heterogeneous uptake of a fluorescently labeled fatty acid by adipocytes in WAT explants from rhesus monkeys. Heterogeneity in the uptake of the fluorescently labeled fatty acid was also observed in adipocytes generated from human stromal vascular cells [5]. These studies employing a fluorescently labeled fatty acid, while not quantitative, supported the idea that the metabolic properties of cultured adipocytes are variable, but they left open the question of whether the same sort of metabolic heterogeneity exists within WAT depots of live animals.

In the case of brown adipocytes, the regulation of metabolic activity has been investigated intensively [6]. However, whether heterogeneity exists in the metabolic properties of individual brown adipocytes has received little attention, and no one has tested whether nutrient uptake is uniform or heterogeneous. In the current studies, we tested whether the uptake of nutrients in brown adipocytes of mice is uniform or heterogeneous. Rather than using fluorescent fatty acids and adipose tissue explants, we injected mice with stable isotope–labeled nutrients and used NanoSIMS [7–11] to quantify uptake of the stable isotopes by brown adipocytes. An attractive feature of this approach is the ability to quantify the heterogeneity of nutrient uptake in brown adipocytes in living animals.

2. Materials and Methods

2.1 Mouse Strains

Wild-type mice and *Gpihbp1*$^{-/-}$ mice [12] (both strain C57BL/6J), 3–4 months of age, were housed in an accredited barrier vivarium with a 12-h light-dark cycle and fed a chow diet. All animal procedures were approved by UCLA's institutional animal care and use committee (the Chancellor's Animal Research Committee).

2.2. Metabolic studies

^{2}H-labeled triglyceride-rich lipoproteins (^{2}H-TRLs) were prepared as described [7]. Briefly, *Gpihbp1*$^{-/-}$ mice were given multiple doses of ^{2}H-labeled mixed fatty acid over 4.5 days. Four hours after the final dose, blood was collected and ^{2}H-TRLs were isolated from the plasma by ultracentrifugation. The ^{2}H/^{1}H ratio in ^{2}H-TRLs was assessed by NanoSIMS; ~25–35% of the hydrogen atoms were ^{2}H.

^{2}H-TRLs (~40 μg triglycerides in a volume of 200 μl) were injected into anesthetized 3–4-month-old wild-type mice [7]. After 1, 2, 15, or 30 min, the mice were sacrificed, perfusion-fixed with 4% *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (carbodiimide) and 0.4% glutaraldehyde in a 0.1 M phosphate buffer [7]. 1-mm^{3} pieces of BAT (trimmed of all WAT) were placed in 4% carbodiimide and 2.5% glutaraldehyde

overnight at 4°C. In other experiments, 3–4-month-old mice were given 80 μl of [^{13}C]fatty acids (1 mg/μl; Cambridge Isotope Laboratories; two doses 12 h apart) or 75 μl of [^{13}C]glucose (1 mg/μl, Cambridge Isotope Laboratories; three doses 12 h apart) by gastric gavage. BAT was harvested 22 h after the final dose of fatty acids or 4 h after the final dose of glucose, and sections were prepared for NanoSIMS as described [7].

2.3 NanoSIMS

Tissue sections were coated with 5 nm of platinum and analyzed with NanoSIMS 50L or NanoSIMS 50 instruments [7, 8]. A 16-KeV $^{133}Cs^{+}$ beam was used to bombard the sample, and secondary electrons (SEs) and secondary ions (*e.g.,* $^{12}C^{14}N–$, $^{1}H–$, $^{2}H–$, $^{12}C–$, $^{13}C–$) were collected [7]. To assess nutrient uptake, regions-of-interest were selected and $^{2}H/^{1}H$ and $^{13}C/^{12}C$ ratios were measured with the OpenMIMS plugin in ImageJ and processed by GraphPad Prism 7.0.

3. Results

^{2}H-TRLs were injected intravenously into wild-type mice. After 1, 15, or 30 min, interscapular BAT was harvested for NanoSIMS analyses (Fig. 1). $^{12}C^{14}N–$ NanoSIMS images were created to visualize tissue morphology, and $^{2}H/^{1}H$ ratio images were useful for defining ^{2}H enrichment in adipocytes. The processing of ^{2}H-TRLs and the subsequent uptake of ^{2}H-labeled lipids by adipocytes was rapid; ^{2}H enrichment in the cytosolic lipid droplets of brown adipocytes was easily detectable 1 min after the ^{2}H-TRL injection. As expected [7], marginated ^{2}H-TRLs were present along the capillary lumen at the 1-min time point but were much less common at the 15-and 30-min time points (Fig. 1). The $^{2}H/^{1}H$ ratio in adipocytes varied by more than fourfold at each of the three time points (n = 30–40 adipocytes/time point) (Fig. 1). Within individual adipocytes, the ^{2}H content within different cytosolic lipid droplets was almost always homogeneous (Fig. 1). $^{2}H/^{1}H$ ratios were measured in cytosolic lipid droplets and in nitrogen-rich regions of the cytoplasm (primarily mitochondria), and those ratios were positively correlated (1 min, $R^2 = 0.56$, $P < 0.0001$; 15 min, $R^2 = 0.58$, $P < 0.0001$; 30 min, $R^2 = 0.33$, $P < 0.0001$). The heterogeneity in ^{2}H uptake in brown adipocytes exceeded that in left ventricular cardiomyocytes (n = 30–40), where ^{2}H enrichment varied by only 1.5-fold.

Heterogeneity in the uptake of ^{2}H-TRL–derived lipids by individual brown adipocytes was very similar in perivascular adipose tissue (PVAT) (Fig. 2). To further explore nutrient uptake in brown adipocytes, mice were given ^{13}C-labeled mixed fatty acids or [^{13}C]glucose by gastric gavage. Interscapular BAT was harvested 22 h after the final dose of the mixed fatty acids or 4 h after the final dose of glucose, and NanoSIMS imaging was performed. We observed heterogeneity in ^{13}C enrichment in brown adipocytes, varying by ~2.3-fold in both the fatty acid and glucose studies (Fig. 3).

4. Discussion

We investigated heterogeneity in fatty acid uptake in brown adipocytes after an intravenous injection of ^{2}H–TRLs. At each of the three time points examined, we observed substantial heterogeneity in ^{2}H enrichment in brown adipocytes—even within adjacent adipocytes. Our

findings are consistent with earlier studies showing heterogenous uptake of fatty acids by adipocytes of rhesus monkey WAT explants [5]. Heterogeneity in ^{13}C enrichment in different brown adipocytes was also observed after administering ^{13}C-labeled mixed fatty acids or [^{13}C]glucose by gastric gavage, although the extent of the heterogeneity was somewhat lower. However, in considering the results of the latter experiments, it is important to keep in mind that the studies were performed nearly two days after administering the first doses of ^{13}C-labeled nutrients, allowing ample time for ^{13}C-labeled nutrients to be converted into other metabolites (*e.g.*, nonessential amino acids) and taken up by cells [7].

The analytical method that we used to assess nutrient uptake, NanoSIMS imaging, is attractive because it allows one to examine and quantify nutrient uptake *in vivo* without relying on lipids containing a fluorescent tag. Also, because of the high resolution of NanoSIMS images, it is possible to assess and quantify stable isotope enrichment in both cytosolic lipid droplets and the lipid droplet–free nitrogen-rich regions of the cytoplasm (primarily mitochondria). A downside of NanoSIMS imaging is that it is very expensive and time-consuming, making it unrealistic to examine many different tissues or a host of genetically modified mouse models. In our experience, NanoSIMS imaging is particularly useful for examining metabolic questions with an anatomical angle—such as in the current study where we examined heterogeneity in nutrient uptake by individual brown adipocytes.

Metabolic heterogeneity in white adipocytes has been documented extensively [1–3], but heterogeneity in brown adipocytes has received much less attention. In future studies, it will be important to define the biochemical basis for the inhomogenous uptake of nutrients. A potential clue is that there is substantial heterogeneity in UCP1 expression in individual brown adipocytes in Sprague-Dawley rats [13]. It would be interesting to determine if similar heterogeneity in UCP1 expression exists in individual adipocytes of mouse BAT, and if so, whether the UCP1 expression correlates inversely with ^{13}C or ^{2}H enrichment after administering nutrients enriched in those isotopes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

NanoSIMS	Nanoscale secondary ion mass spectrometry
TRLs	triglyceride-rich lipoproteins
WAT	white adipose tissue
BAT	brown adipose tissue

cAMP	cyclic adenosine monophosphate
UCP1	uncoupling protein 1

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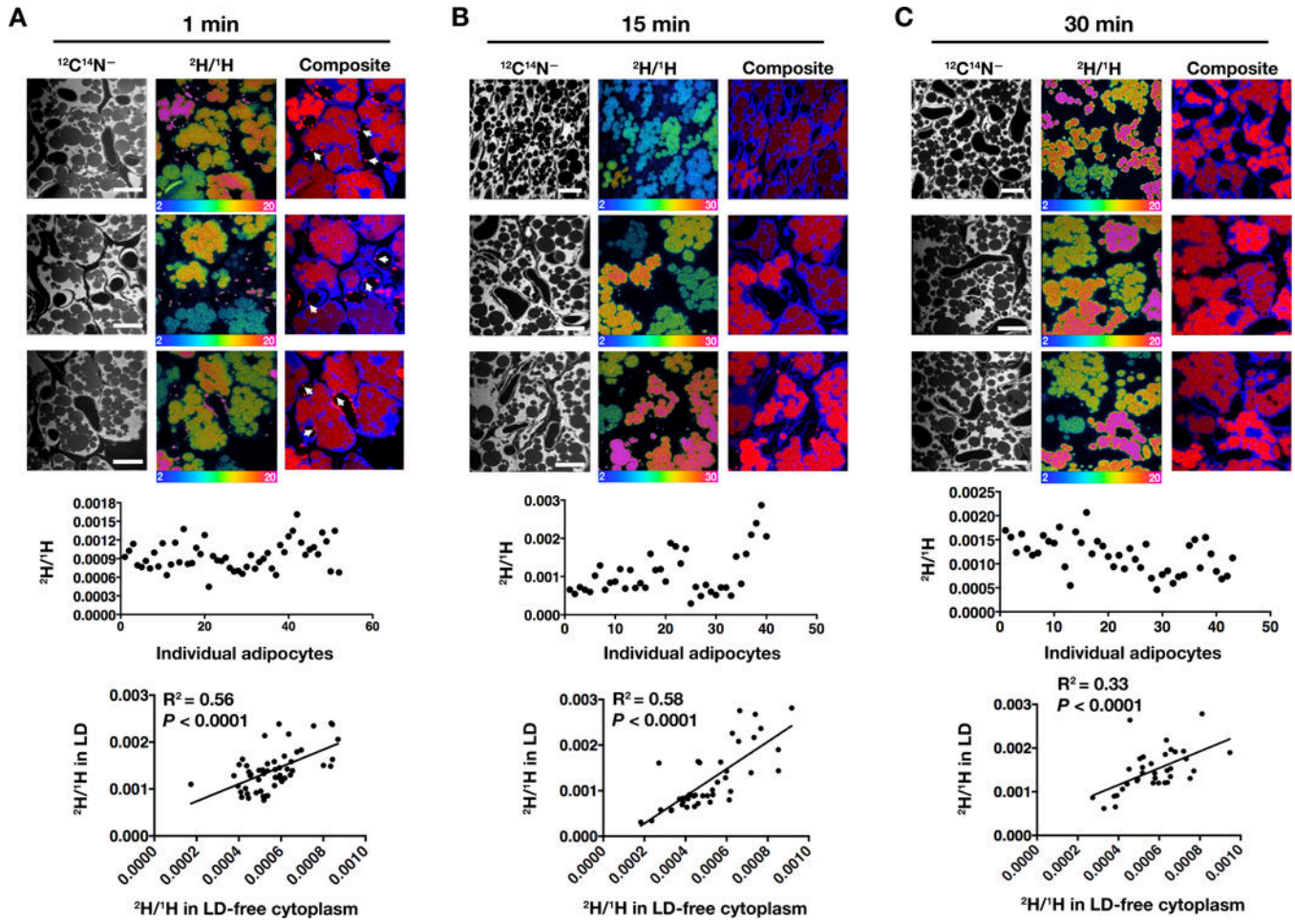

Fig. 1.

Heterogeneity in the uptake of deuterated fatty acids (derived from the lipolytic processing of ^{2}H-TRLs) in the adipocytes of interscapular BAT. Four-month-old wild-type mice were fasted for 4 h and then injected with 200 μl of ^{2}H-TRLs (see *Methods*). After 1 min (A), 15 min (B), or 30 min (C), the mice (1 mouse/time point) were perfusion-fixed with carbodiimide and glutaraldehyde, and tissue sections were prepared for NanoSIMS analysis. $^{12}C^{14}N–$ NanoSIMS images were generated to visualize tissue morphology; $^{2}H/^{1}H$ ratio images or composite $^{12}C^{14}N–$ (blue) and $^{2}H/^{1}H$ ratio (red) images revealed ^{2}H enrichment in adipocyte lipid droplets and ^{2}H-TRLs that had marginated along capillaries (arrows). The scale shows the $^{2}H/^{1}H$ ratio multiplied by 10,000. The ^{2}H natural abundance, relative to ^{1}H, is 0.000156. Scale bars, 10 μm. The $^{2}H/^{1}H$ ratios in all adipocytes (n = 30–40/time point) were quantified and plotted as a scatter plot. Also plotted is the correlation between $^{2}H/^{1}H$ ratios in cytoplasmic lipid droplets (LD) and nitrogen-rich LD-free regions of the cytoplasm (primarily mitochondria).

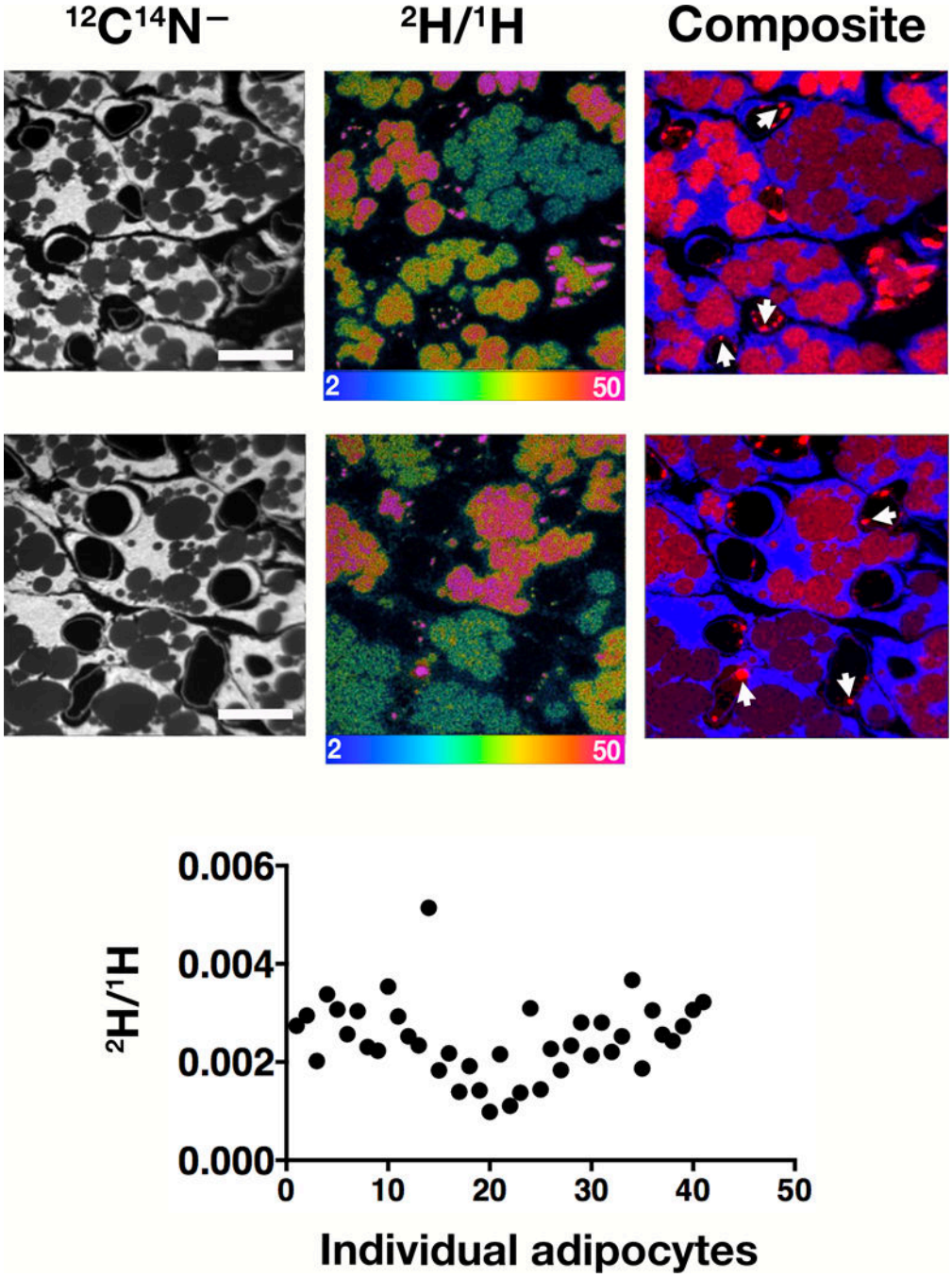

Fig. 2.

Heterogeneity in the uptake of deuterated fatty acids (derived from the lipolytic processing of ^{2}H-TRLs) in the adipocytes of perivascular adipose tissue (PVAT). A four-month-old wild-type mouse was fasted for 4 h and then injected with 200 μl ^{2}H-TRLs. After 1 min, the mouse was euthanized; perfusion-fixed with carbodiimide/glutaraldehyde; and sections of PVAT prepared to generate NanoSIMS images, exactly as in Fig. 1. Arrows point to marginated lipoproteins in capillaries.

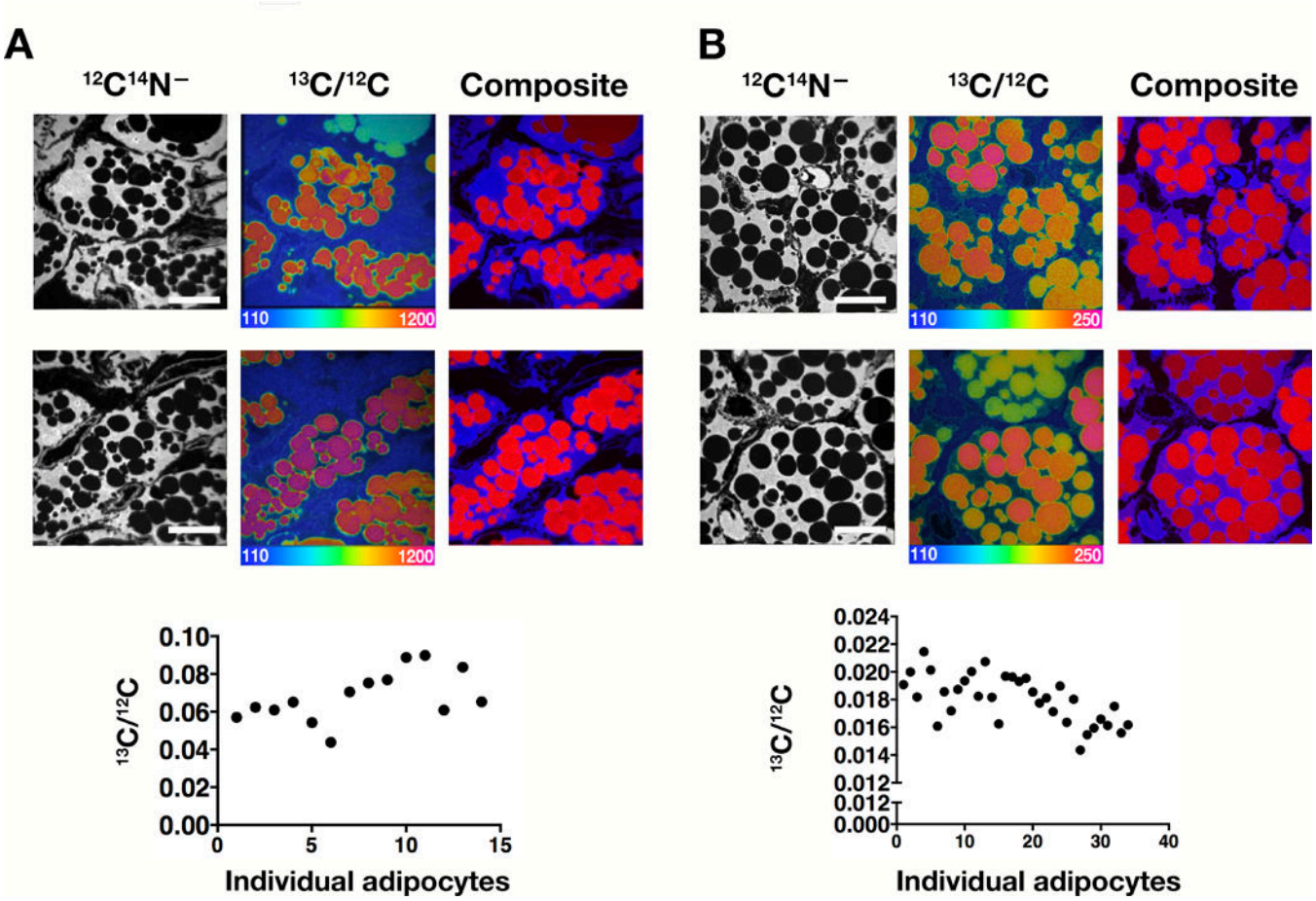

Fig. 3.

Heterogeneity of ^{13}C enrichment in interscapular brown adipocytes following the administration of [^{13}C]fatty acids or [^{13}C]glucose. (A) ^{13}C-labeled mixed fatty acids were administered to a 4-month-old wild-type mouse by gastric gavage (see *Methods*). 22 h after the final dose, BAT was harvested and prepared for NanoSIMS imaging. (B) [^{13}C]glucose was administered to a 4-month-old wild-type mouse by gastric gavage (see *Methods*). 4 h after the final dose, BAT was harvested and prepared for NanoSIMS imaging. For panels B and C, $^{12}C^{14}N$– images were generated to visualize tissue morphology, and the $^{13}C/^{12}C$ ratio images or composite $^{12}C^{14}N$– (blue) and $^{13}C/^{12}C$ (red) images revealed heterogeneity of nutrient uptake. The scale shows the $^{2}H/^{1}H$ and $^{13}C/^{12}C$ ratio multiplied by 10,000. Scale bars, 10 μm. The average $^{2}H/^{1}H$ and $^{13}C/^{12}C$ ratio in each adipocyte (n = 15–40) was quantified and plotted as a scatter plot.