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Dissociation between Brown Adipose Tissue ^{18}F-FDG Uptake and Thermogenesis in Uncoupling Protein 1 Deficient Mice

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ABSTRACT

^{18}F-FDG PET imaging is routinely used to investigate brown adipose tissue (BAT) thermogenesis which requires mitochondrial uncoupling protein 1 (UCP1). It remains uncertain whether BAT ^{18}F-FDG uptake reliably tracks UCP1-mediated heat production.

METHODS: UCP1 knockout (KO) and wild-type (WT) mice received the selective β3 adrenergic receptor agonist CL 316, 243 (1mg/kg) and underwent metabolic cage, infrared thermal imaging and ^{18}F-FDG PET/magnetic resonance imaging (MRI) experiments. Primary brown adipocytes were additionally examined for their bioenergetics by extracellular flux analysis as well as their uptake of 2-deoxy-^{3}H-glucose.

RESULTS: In response to CL 316, 243 treatments, oxygen consumption and BAT thermogenesis were diminished in UCP1 KO mice but BAT ^{18}F-FDG uptake was fully retained. UCP1 KO brown adipocytes exhibited defective induction of uncoupled respiration whereas their glycolytic flux and 2-deoxy-^{3}H-glucose uptake rates were largely unaffected.

CONCLUSION: Increased BAT^{18}F-FDG uptake can occur independently of UCP1 function.

INTRODUCTION

In human PET imaging studies, BAT ^{18}F-FDG uptake has been shown to be markedly stimulated by controlled cold exposure *(1,2)* and agonism of β3 adrenergic receptors (3). Similar findings have been made in small-animal PET imaging studies performed on mice *(4,5)*.

Mitochondrial UCP1 endows both human and mouse brown adipocytes with thermogenic capacity *(6)*. It is widely held that by BAT^{18}F-FDG uptake provides an indirect measure of thermogenesis. We therefore sought to formally test this assertion by performing ^{18}F-FDG PET/MRI experiments on UCP1 deficient mice that exhibit defective BAT thermogenesis and uncoupled respiration in response to the selective β3 adrenergic receptor agonist CL 316, 243 *(7, 8)*.

METHODS

Animals

Experiments were performed on female WT C57BL/6J mice and UCP1 KO littermates (Jackson Laboratory) aged 3 to 9 mo and were all approved by the Institutional Animal Care and Use Committee at the University of Leipzig (TVV 63/13 and TVV 04/12). Animals were housed in an incubator (Memmert GmbH & Co. KG) set at thermoneutrality (30 ºC) and 60% humidity on a 12-h light/dark cycle and had free access to a high fat high sugar diet (Sniff GmbH) to potentiate thermogenic responses to adrenergic stimulation *(9)* unless otherwise indicated. In line with the findings of Feldmann et al., UCP1 KO mice housed under these conditions consistently showed higher body weights than WT littermates (Table 1).

Indirect Calorimetry

Animals were transferred to metabolic cages (TSE Systems) immediately following intraperitoneal injection of the selective β3 adrenergic receptor agonist CL 316, 243 (1mg/kg) (Tocris) *(7)* and oxygen consumption was measured for 3 hr by indirect calorimetry.

Core Body Temperature Measurements

Core body temperature was measured using a rectal probe (Thermalert) at baseline and at 15 min intervals for 1 hr following intraperitoneal injection of CL 316, 243 (1mg/kg) *(8)*.

Infrared Thermal Imaging

Infrared thermal imaging *(9)* was performed using a VarioCAM thermal camera (InfraTec GmbH). Each mouse was placed on a cage top at a fixed distance away from the camera lens. Serial one second images (10 Hz) were taken in triplicate at baseline and at 15 min intervals for 1 hr following intraperitoneal injection of CL 316, 243 (1mg/kg). For analysis, a constantly sized circular region of interest was drawn over BAT and the average temperature was recorded.

PET/MRI

Small-animal PET/MRI (Mediso Medical Imaging Systems) was performed as previously described *(10)*. Overnight fasted mice received intraperitoneal injections of either CL 316, 243 (1mg/kg) *(4)* or 0.9% saline along with an intraperitoneal injection of 18.8 ± 0.4 MBq ^{18}F-FDG (Supplier: Department of Nuclear Medicine, University Hospital Leipzig). Animals were then returned to their home cages for 45 min and subsequently anaesthetized with Isoflurane (1.8%, 0.35 L/min) delivered in a 60% oxygen/40% air mixture (MCQ Instruments) and transferred to the PET/MRI scanner. A 15 min static PET scan was initiated, during which animals were maintained at 37^{o}C under Isoflurane anesthesia. For analysis, mean standardized uptake values of ^{18}F-FDG by BAT were calculated.

Cellular Bioenergetics and 2-deoxy-^{3}H-glucose Assay

Stromal vascular cells were isolated from interscapular BAT and differentiated as previously described *(11)*. On day 7 of differentiation, mature brown adipocytes were washed twice with assay medium (XF Dulbecco's Modified Eagle Medium supplemented with 25 mM glucose, 2 mM pyruvate, and 4% Bovine Serum Albumin (w/v)), followed by incubation at 37 °C without CO_2 for 20 min. Cells were transferred to an XF96 Extracellular Flux Analyzer (Seahorse Bioscience) and after 5 basal values, 5 µg/mL oligomycin was injected to induce uncoupled respiration followed by an injection of 100 µM 2-,4-dinitrophenol. To correct for non-mitochondrial respiration and non-glycolytic sources of changes in extracurricular acidification rate, a cocktail of 2.5 µM rotenone, 2.5 µM antimycin A and 2.5 µM 2-deoxyglucose was

injected. For analysis, basal values were averaged as were the 3 values immediately after oligomycin treatment and the 3 values 1 hr after oligomycin treatment.

For the 2-deoxy-^{3}H-glucose uptake assay, mature brown adipocytes were cultivated overnight in serum-free medium and were stimulated with 1 μM CL 316, 243 (Sigma-Aldrich GmbH) *(12)* or distilled water followed by incubation at 37°C in 5% CO_2 for 110 min. Cells were washed once with pre-warmed phosphate buffered saline, then 0.5μCi/ml 2-deoxy-^{3}H-glucose and 100 μM 2-deoxy-glucose was added followed by incubation at 37°C in 5% CO_2 for 10 min. After washing with ice-cold phosphate buffered saline, cells were lysed with radioimmunoprecipitation assay buffer and transferred to a liquid scintillation analyzer (PerkinElmer) for 3 hr. Uptake rates of 2-deoxy-^{3}H-glucose were normalized to protein concentration.

Statistics

Two-way analysis of variance with Sidak's post-hoc test was used to assess differences between groups (GraphPad Software Inc). *P* values less than 0.05 were considered as significant.

RESULTS

The increase in whole body oxygen consumption caused by CL 316, 243 treatment was blunted in UCP1 KO compared to WT mice ($F_{1, 90} = 554.8, P < 0.0001$) (Fig. 1A) as was the rise in core body temperature ($F_{1, 35} = 10.77, P = 0.0023$) (Fig. 1B).

Representative thermal images are presented in Figure 2A. Unlike UCP1 KO mice, WT mice showed an increase in interscapular BAT temperature in response to CL 316, 243 treatment ($F_{1, 30} = 18.53, P = 0.0002$) (Fig. 2B).

Representative ^{18}F-FDG PET/MRI data are presented in Figure 3A. Both WT and UCP1 KO mice showed an increased mean standardized uptake value for^{18}F-FDG in BAT in response to CL 316, 243 treatment relative to vehicle treatment ($P < 0.0001$) (Fig. 3B) with no significant effect of genotype found ($F_{1, 14} = 0.17, P = 0.68$).

Extracellular flux analysis of cultured primary brown adipocytes revealed that one hour after oligomycin treatment, reporting induction of uncoupled respiration *(13)*, oxygen consumption rates were significantly lower for UCP1 KO compared to WT cells (Fig. 4A) ($P < 0.001$). In contrast, extracellular acidification rates, reporting glycolytic activity *(13)*, tended to be

similar between UCP1 KO and WT cells at this time point after oligomycin treatment (Fig 4B) (*P = 0.07)*. Both WT and UCP1 KO brown adipocytes showed an increase in 2-deoxy-^{3}H-glucose uptake in response to CL 316, 243 treatment compared to vehicle (*P = 0.01 and P < 0.0001,* respectively) (Fig. 4C) with no significant effect of genotype found ($F_{1, 12} = 0.00045$, $P = 0.97$).

DISCUSSION

The *in vivo* and *in vitro* findings from the present study demonstrate that glucose metabolism can increase in brown adipocytes despite defects in UCP1-mediated uncoupled respiration and heat production.

In mouse primary brown adipocytes treated with CL 316,243, glucose is transported into the cell and through the action of diacylglycerol acyltransferase 2, feeds into specialized lipid droplet pools which are simultaneously hydrolyzed (*14*). The released free fatty acids are then either rapidly oxidized or activate UCP1 in mitochondria (*14*). Our findings of similar β3 adrenergic receptor stimulated ^{18}F-FDG/2-deoxy-^{3}H-glucose uptake between WT and UCP1 KO brown adipocytes suggest that UCP1 (thermogenic) function does not provide feedback, on the initial step at least, of this concerted process.

Noradrenaline treatment was previously found not to stimulate 2-deoxy-^{3}H-glucose uptake by BAT in male UCP1 KO mice (*15*). The discordance with the findings from the present study could be explained by our use of female UCP1 KO mice. Indeed, cold-stimulated ^{18}F-FDG uptake by BAT is negated in male UCP1 KO mice but not in female counterparts (*16*).

It is unlikely that the low BAT ^{18}F-FDG uptake in the present study is diet related (*17*). This is because we found in preliminary experiments performed on high-fat high-sugar fed mice a much higher amount of BAT ^{18}F-FDG uptake when animals were housed and scanned at room temperature (22 °C). This suggests that experimental conditions even only mildly below thermoneutrality (30 °C for mice) *(8)* can significantly stimulate BAT. Therefore, studies under thermoneutral conditions are optimal as the low BAT activity constitutes a true basal state allowing the effects novel stimulators of BAT to be examined in isolation.

In summary, BAT ^{18}F-FDG uptake and UCP1-mediated heat production can be dissociated. Additional techniques that measure BAT thermogenesis apart from ^{18}F-FDG PET imaging and blood flow measurements (*18*) are required in model systems where UCP1 is expected not to be functional. As UCP1 deficiency is not the standard physiological scenario in humans, caution is however warranted when translating our findings.

DISCLOSURE

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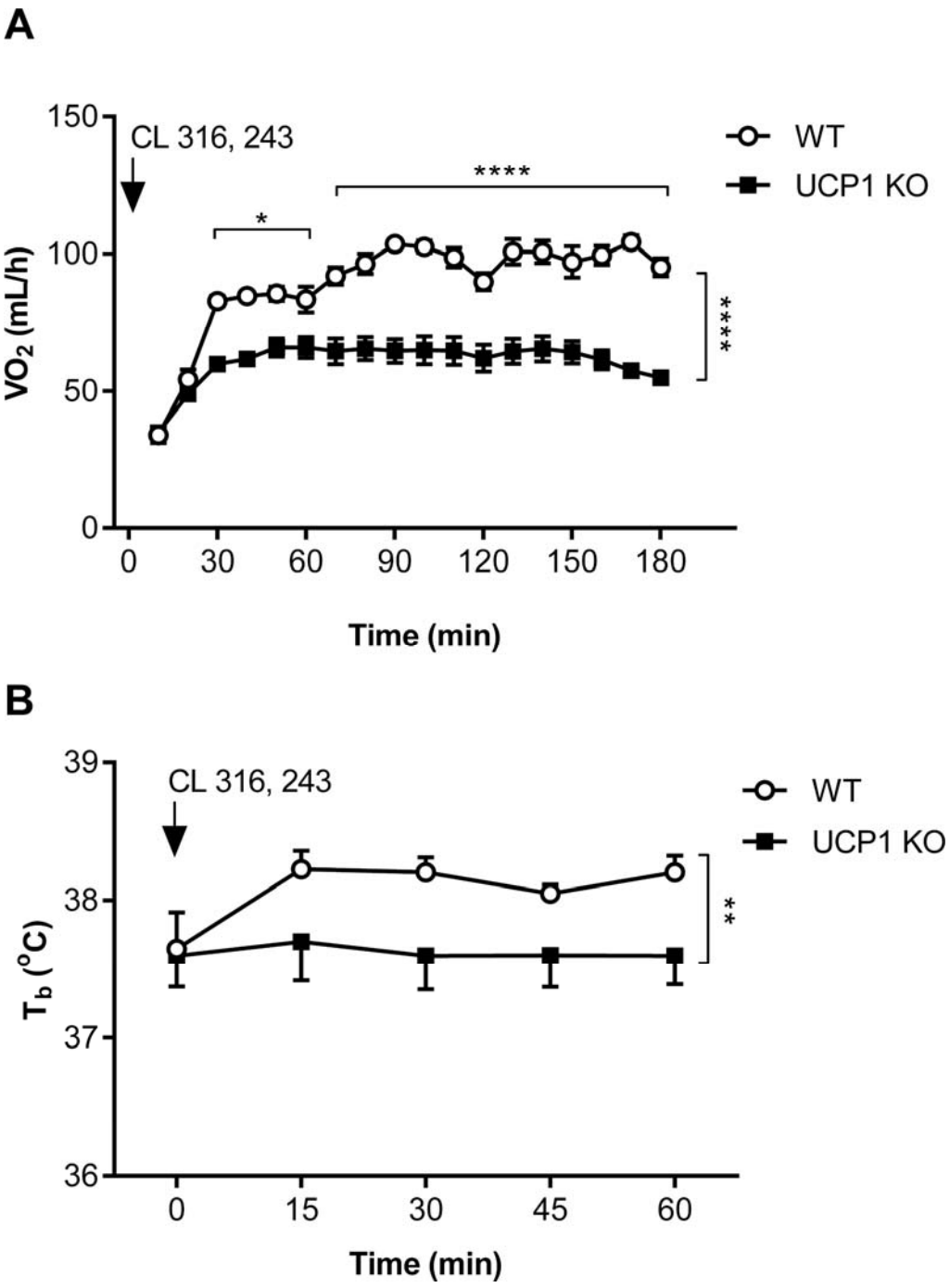

FIGURE 1.

(A) Diminished oxygen consumption and (B) core body temperature (T_b) responses in UCP1 KO mice. Data points represent mean ± SEM of 3 to 5 animals per group. **P < 0.05, **P < 0.01, and ****P < 0.0001*.

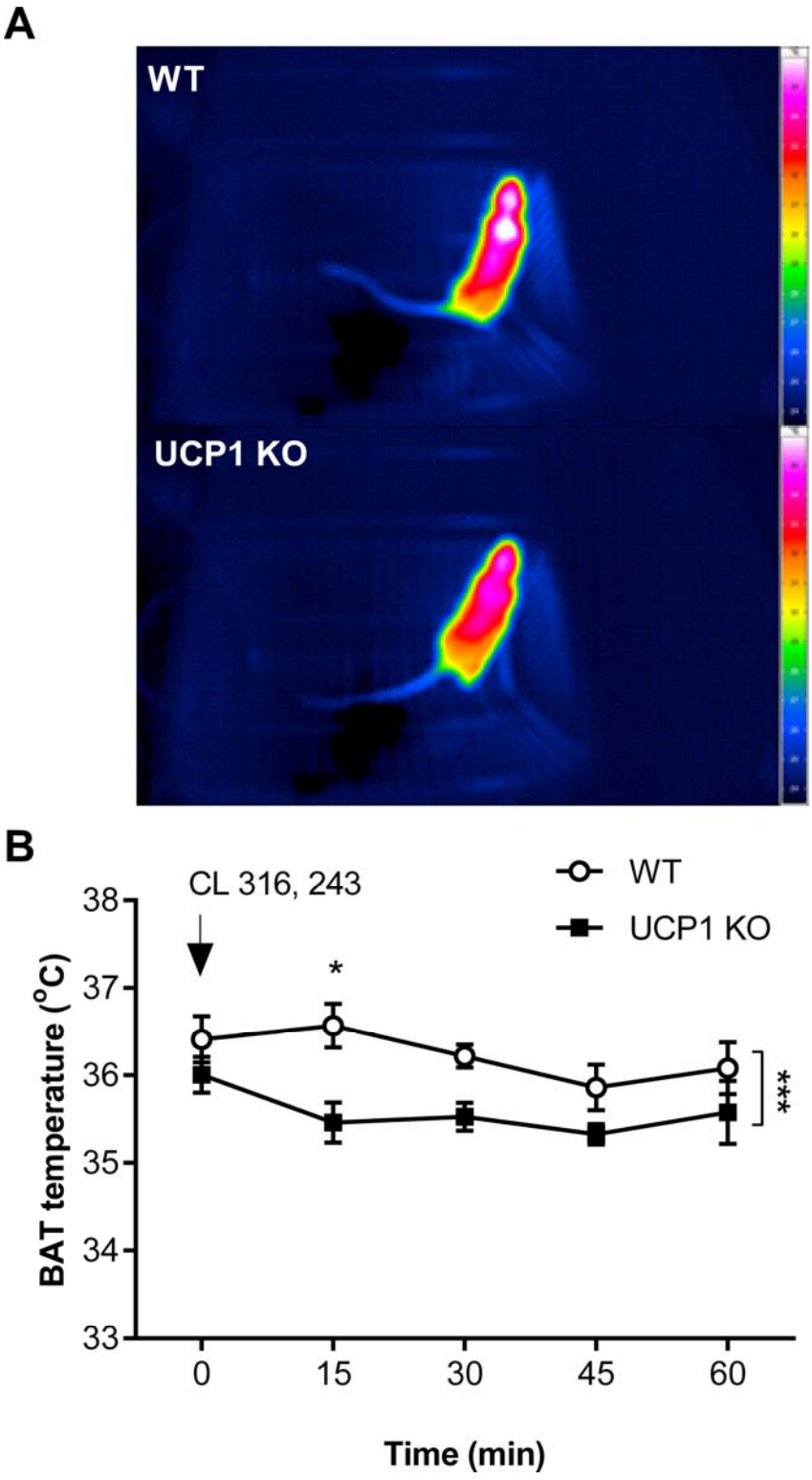

FIGURE 2.

(A) Representative thermal images of WT (top) and UCP1 KO (bottom) mice taken 15 min after CL 316, 243 treatments. (B) Defective BAT thermogenesis in UCP1 KO mice. Data points represent mean ± SEM of 4 animals per group. *$P < 0.05$ and ***$P < 0.001$.

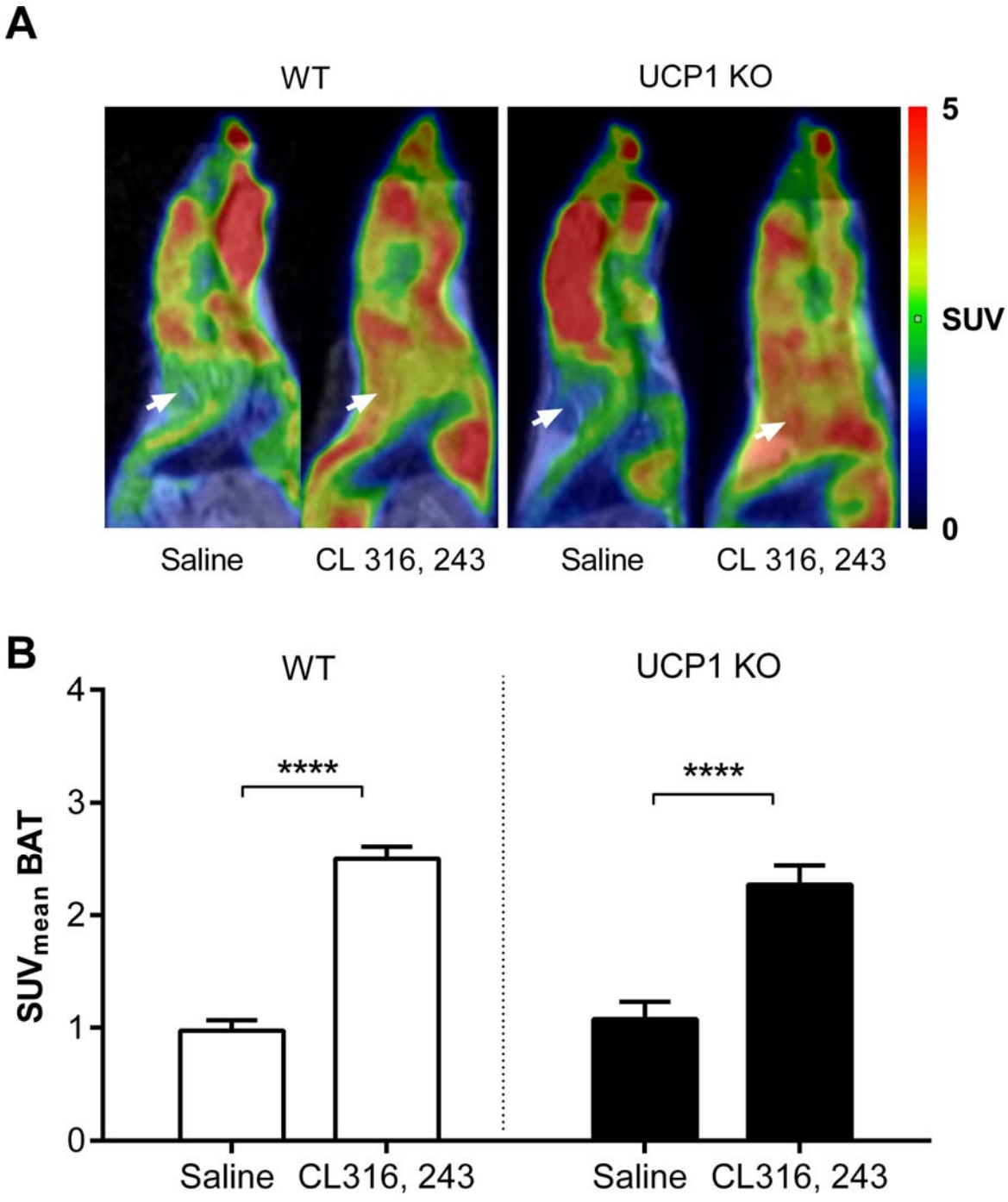

FIGURE 3.

(A) Representative PET/MRI data of WT and UCP1 KO mice taken 45-60 min after CL 316, 243/saline and ^{18}F-FDG injections. The white arrows in each image point to interscapular BAT. (B) ^{18}F-FDG uptake by BAT is retained in UCP1 KO mice. Bars represent mean ± SEM of 4 to 5 animals per group. SUV_{mean} = mean standardized uptake value. ****$P < 0.0001$.

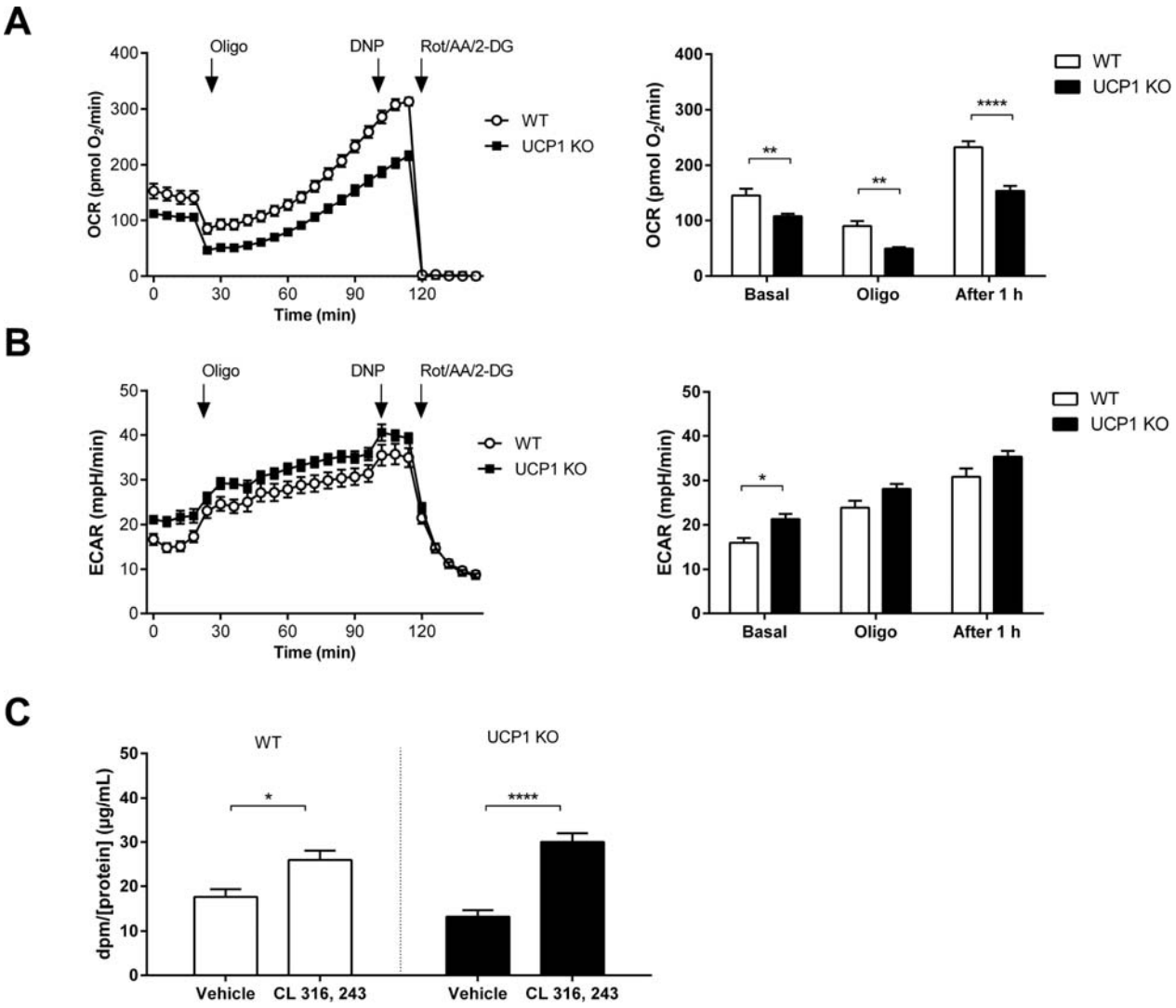

FIGURE 4.

Induction of uncoupled respiration was diminished (A) but glycolysis (B) and glucose uptake (C) was normal in UCP1 KO brown adipocytes. Data points represent mean ± SEM of 4 to 6 cultures per group. DNP = dinitrophenol, Rot = rotenone, AA = antimycin A, 2-DG = 2-deoxyglucose, ECAR = extracellular acidification rate, dpm = dose per minute. **P < 0.05, **P < 0.01, and ****P < 0.0001*.

	WT body weights	UCP1 KO body weights	
Indirect calorimetry	25.3 ± 0.5 g (n = 3)	29.3 ± 0.3 g (n = 4)	*P = 0.0006*
Rectal measurements	23.8 ± 0.3 g (n = 4)	27.3 ± 0.6 g (n = 5)	*P = 0.0023*
Thermal imaging	25.3 ± 0.5 g (n = 4)	28.3 ± 0.3 g (n = 4)	*P = 0.0014*
PET/MRI (saline)	20.6 ± 0.2 g (n = 4)	24.3 ± 0.8 g (n = 4)	*P = 0.0014*
PET/MRI (CL 316, 243)	20.3 ± 0.8 g (n = 5)	24.5 ± 0.4 g (n = 5)	*P = 0.0015*

Table 1.

UCP1 KO mice weighed more than their WT littermates at the time of experiments. Values represent mean ± SEM. *P* values were obtained from unpaired two-tailed *t* tests.