Brown adipose tissue takes up plasma triglycerides mostly after lipolysis

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Abstract **Brown adipose tissue (BAT) produces heat by burning TGs that are stored within intracellular lipid droplets and need to be replenished by the uptake of TG-derived FA from plasma. It is currently unclear whether BAT takes up FA via uptake of TG-rich lipoproteins (TRLs), after lipolysis-mediated liberation of FA, or via a combination of both. Therefore, we generated glycerol tri[^{3}H]oleate and [^{14}C]cholesteryl oleate double-labeled TRL-mimicking particles with an average diameter of 45, 80, and 150 nm (representing small VLDL to chylomicrons) and injected these intravenously into male C57Bl/6J mice. At room temperature (21°C), the uptake of ^{3}H-activity by BAT, expressed per gram of tissue, was much higher than the uptake of ^{14}C-activity, irrespective of particle size, indicating lipolysis-mediated uptake of TG-derived FA rather than whole particle uptake. Cold exposure (7°C) increased the uptake of FA derived from the differently sized particles by BAT, while retaining the selectivity for uptake of FA over cholesteryl ester (CE). At thermoneutrality (28°C), total FA uptake by BAT was attenuated, but the specificity of uptake of FA over CE was again largely retained. Altogether, we conclude that, in our model, BAT takes up plasma TG preferentially by means of lipolysis-mediated uptake of FA.**—Khedoe, P. P. S. J., G. Hoeke, S. Kooijman, W. Dijk, J. T. Buijs, S. Kersten, L. M. Havekes, P. S. Hiemstra, J. F. P. Berbée, M. R. Boon, and P. C. N. Rensen. **Brown adipose tissue takes up plasma triglycerides mostly after lipolysis.** ***J. Lipid Res.*** **2015.** 56: **51–59.**

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Brown adipose tissue (BAT) is an important player in energy homeostasis due to its ability to combust energy toward heat by virtue of the presence of uncoupling protein 1 (UCP1), a process called nonshivering thermogenesis (1). The most well-known trigger for activation of BAT is cold, which increases sympathetic outflow from the hypothalamic temperature center toward BAT. Here, nerve endings release noradrenalin that binds to adrenergic receptors on the brown adipocyte membrane (2). Activation of an intracellular signaling cascade subsequently leads to a rapid induction of intracellular lipolysis, mediated by adipose triglyceride lipase (ATGL), hormone sensitive lipase, and monoglyceride lipase, resulting in release of FA from TG-filled lipid droplets (3). FAs are directed to the mitochondria where they either allosterically activate UCP1 present on the inner membrane of the mitochondria or undergo β-oxidation within the mitochondrial matrix (2). Upon activation, UCP1 dissipates the proton gradient across the inner mitochondrial membrane that is generated by the respiratory chain, resulting in production of heat. Of note, FAs used for activation of UCP1 and β-oxidation appear to be mainly derived from intracellular TG stores, rather than from directly internalized FA, as mice that lack ATGL exhibit defective thermogenesis (4). Therefore, replenishment of intracellular TG stores within the brown adipocyte is essential for nonshivering thermogenesis in BAT.

Replenishment of intracellular TG stores is mediated via three mechanisms: uptake of glucose followed by de novo lipogenesis, uptake of albumin-bound FA, and uptake of triglyceride-rich lipoprotein (TRL)-derived FA from the

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Abbreviations: [^{14}C]CO, [^{14}C]cholesteryl oleate; [^{3}H]COE, [^{3}H]cholesteryl oleoyl ether; [^{3}H]TO, glycerol tri[^{3}H]oleate ([^{3}H]triolein); BAT, brown adipose tissue; TRL, triglyceride-rich lipoprotein; UCP1, uncoupling protein 1; WAT, white adipose tissue.

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plasma followed by incorporation of FA within TG (2, 3, 5). Circulating TRLs [i.e., VLDL (particle size 40–80 nm) and chylomicrons (particle size 100–500 nm)] are the main source for FA stored as TG in BAT (3).

Only recently, BAT appeared as a major player in plasma TG clearance. Bartelt et al. (5) showed that 24 h of cold exposure markedly enhanced clearance of glycerol tri[^{3}H] oleate ([^{3}H]TO)-labeled TRLs, which was specifically mediated by BAT (5, 6). The authors suggested that upon cold exposure, BAT internalized TG from chylomicron-sized TRL-like particles (~250 nm) via whole particle uptake. However, they also demonstrated that BAT activation by cold was accompanied by enhanced expression of *Lpl* and *Cd36*, and the presence of both appeared critical for the uptake of TG (5, 6). Interestingly, the critical involvement of LPL and CD36 suggests lipolysis-mediated uptake of TRL-derived FAs rather than whole particles as would also occur in skeletal muscle, heart, and white adipose tissue (WAT) (7). The formed remnant particles, depleted of TG, are subsequently taken up by the liver through an interaction of apoE with the low density lipoprotein receptor (8).

The aim of the present study was to further investigate how BAT takes up lipoprotein-derived FA from the circulation, examining the importance of selective delipidation of circulating TRL by LPL and whole particle uptake of TRL. To this end, we assessed the uptake of FA into BAT by injecting [^{3}H]TO and [^{14}C] cholesteryl oleate ([^{14}C]CO) double-labeled TRL-mimicking particles with diameters ranging from small VLDL to chylomicrons (45–150 nm) in mice, while modulating the activity of BAT using various ambient temperatures (7, 21, and 28°C).

MATERIALS AND METHODS

Animals and diet

For all studies, 8- to 10-week-old male C57Bl/6J mice (Jackson Laboratory, Bar Harbor, ME) were used. Mice were housed in conventional cages with a 12:12 h light-dark cycle and had free access to chow food and water. All mouse experiments were performed in accordance with the Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals and have received approval from the Animal Ethical Committee (Leiden University Medical Center, Leiden, the Netherlands).

Acclimation to ambient temperature

Mice were single-housed 1 week prior to the experiment at an environmental temperature of 21°C. Subsequently, they were randomized based on fasting plasma TG levels, total cholesterol (TC) levels, and body weight in two groups that were exposed to an ambient temperature of 7°C or 21°C for 24 h. During the last 4 h, mice were fasted before performing a terminal kinetic experiment with TRL-mimicking particles (see below). For the first experiment, mice in each temperature group were divided into three groups that received [^{3}H]TO- and [^{14}C]CO-labeled TRL-mimicking particles of different sizes (average 45, 80, or 150 nm, n = 6 per group). An additional set of mice received (nondegradable) [^{3}H]cholesteryl oleoyl ether ([^{3}H]COE)-labeled TRL-mimicking particles of 150 nm. To investigate TG kinetics under thermoneutral conditions, mice were randomized into two groups that were exposed for 4 h to an ambient temperature of 21°C or 28°C, while being fasted, prior to the kinetic experiment. For this experiment, mice in each temperature group were divided into three groups that received double-labeled TRL-mimicking particles of different size (average 45, 80, or 150 nm, n = 6 per group).

Plasma parameters

At randomization and prior to the clearance experiment, a blood sample was collected from the tail vein of 4 h fasted mice into capillaries. Plasma was assayed for TG and TC using enzymatic kits from Roche Diagnostics (Mannheim, Germany).

Preparation of radiolabeled TRL-mimicking emulsion particles

Radiolabeled TRL-mimicking emulsion particles were prepared from 100 mg of total lipid including triolein (70 mg), egg yolk phosphatidylcholine (22.7 mg), lysophosphatidylcholine (2.3 mg), cholesteryl oleate (3.0 mg), and cholesterol (2.0 mg), with addition of [^{3}H]TO (100 μCi) and [^{14}C]CO (10 μCi) (9). In addition, TRL-mimicking particles were prepared with the nondegradable label [^{3}H]COE (40 μCi). Sonification was performed using a Soniprep 150 (MSE Scientific Instruments, UK) that is equipped with a water bath for temperature (54°C) maintenance, at 10 μm output (9). The emulsion was fractionated by consecutive density gradient ultracentrifugation steps in a Beckman SW 40 Ti rotor. After centrifugation for 27 min at 20,000 rpm at 20°C, an emulsion fraction containing chylomicron-like particles (average size 150 nm) was removed from the top of the tube by aspiration and replaced by NaCl buffer (1.006 g/ml). After a subsequent centrifugation step for 27 min at 40,000 rpm, large VLDL-like particles (average size 80 nm) were obtained in a similar manner. A third centrifugation step for 3 h at 40,000 rpm yielded small VLDL-like particles (average size 45 nm). The average size of the particles has previously been validated in numerous studies by means of photon correlation spectroscopy, as initially described (10). Characterization of emulsion fractions was done by determination of TG concentration (as described in Plasma Parameters) and radioactivity. Emulsions were stored at 4°C under argon and used for in vivo kinetic experiments within 5 days following preparation.

In vivo clearance of radiolabeled TRL-mimicking emulsion particles

To study the in vivo clearance of radiolabeled TRL-mimicking emulsion particles, mice were fasted for 4 h and injected intravenously with 200 μl of emulsion particles (0.2 mg TG per mouse). Blood samples were taken from the tail vein at 2, 5, 10, and 15 min after injection to determine the plasma decay of either [^{3}H]TO and [^{14}C]CO or [^{3}H]COE. Plasma volumes were calculated as 0.04706 × body weight (g) (11). After taking the last blood sample, mice were euthanized by cervical dislocation and perfused with ice-cold PBS containing 10 U/ml heparin via the heart to remove blood and noninternalized TRL-mimicking particles from the organs. Subsequently, the liver, heart, spleen, hindlimb muscle, gonadal WAT, subcutaneous WAT, and interscapular BAT were collected. Organs were dissolved overnight at 55°C in Tissue Solubilizer (Amersham Biosciences, Roosendaal, the Netherlands), and ^{3}H and ^{14}C activity were quantified. Uptake of [^{3}H]TO-, [^{14}C]CO-, and [^{3}H]COE-derived radioactivity by the organs was expressed per gram wet tissue weight.

RNA purification and quantitative RT-PCR

RNA was extracted from snap-frozen mouse tissues (~25 mg) using Tripure RNA Isolation reagent (Roche) according to the

manufacturer's protocol. Total RNA (1–2 μg) was reverse transcribed using Moloney Murine Leukemia Virus (M-MLV) Reverse Transcriptase (Promega) for quantitative RT-PCR (qRT-PCR) according to the manufacturer's instructions to produce cDNA. mRNA expression was normalized to *β2-microglobulin* and *36b4* mRNA content and expressed as fold change compared with control mice using the ΔΔCT method. The primers sequences used are listed in **Table 1**.

Statistical analysis

Differences between groups were determined using unpaired two-tailed Student's tests with the SPSS 20.0 software package for Windows (SPSS, Chicago, IL). Differences at $P < 0.05$ were considered statistically significant. Data are presented as mean ± SEM.

RESULTS

BAT takes up FA after lipolysis-mediated liberation from TRL-mimicking particles at 21°C

To study the mode of FA uptake by BAT, we generated [^{3}H]TO and [^{14}C]CO double-labeled TRL-mimicking particles with an average diameter of 45, 80, and 150 nm (i.e., representing small VLDL, large VLDL, and chylomicrons, respectively). These particles allowed us to follow the uptake of FA ([^{3}H]oleate) and the remnant core ([^{14}C]CO]) simultaneously. The clearance and distribution of the radiolabels was determined in mice that were exposed to regular room temperature (21°C), cold (7°C), or thermoneutrality (28°C) prior to the kinetic experiment.

In mice exposed to the regular temperature of 21°C, the plasma clearance of [^{3}H]TO was faster ($t_{1/2}$ 2.1, 1.6, and 1.7 min) (**Fig. 1A–C**) than that of [^{14}C]CO ($t_{1/2}$ 4.2, 2.1, and 2.9 min) (Fig. 1D–F) for particles of 45, 80, and 150 nm, respectively. This suggests that for all particles the uptake of FA by organs precedes the uptake of cholesteryl esters, suggestive of peripheral LPL-mediated TG hydrolysis and uptake of [^{3}H]oleate by organs, with generation of [^{14}C]CO-labeled core remnants that are subsequently cleared by the liver. Indeed, in metabolic tissues that express LPL (i.e., heart, muscle, WAT, and BAT), the uptake of ^{3}H-activity (**Fig. 2A–C**) was higher than that of ^{14}C-activity (Fig. 2D–F); whereas in the liver the uptake of ^{14}C-activity was much higher than that of ^{3}H-activity. Remarkably, the specific uptake of ^{3}H-activity by BAT exceeded that of all other organs and was much higher (28, 28, and 20% of injected dose/g) (Fig. 2A–C) than the uptake of ^{14}C-activity (2, 3, and 4% of injected dose/g) (Fig. 2D–F), indicating lipolysis-mediated uptake of TG-derived FA with little uptake of core remnants by BAT at 21°C.

Cold exposure enhances lipolysis-mediated FA uptake from TRL-mimicking particles by BAT

Housing mice for 24 h at 7°C accelerated the plasma clearance of [^{3}H]TO for particles of 45, 80, and 150 nm as compared with 21°C ($t_{1/2}$ = 1.3, 1.5, and 1.6 min, respectively) (Fig. 1A–C), due to a lipolysis-mediated enhanced uptake of ^{3}H-activity by BAT (+139%, $P < 0.01$; +111%, $P < 0.05$; and +150%, $P < 0.001$) (Fig. 2A–C). Accordingly, 24 h of cold exposure resulted in a 43% reduction in plasma TG levels (21°C: 0.58 ± 0.05 vs. 7°C: 0.33 ± 0.02 mM, $P < 0.001$). This was accompanied by increased expression of *Lpl* and *Cd36* (1.7-fold and 2.1-fold, $P < 0.001$), which is in line with increased lipolysis of TG from TRL-mimicking particles followed by uptake of liberated FA by BAT. Likewise, cold exposure accelerated the plasma decay of [^{14}C]CO (Fig. 1D–F), which was accompanied by an increased retention of ^{14}C-label by BAT (+168%, $P < 0.05$; +181%, $P < 0.001$; and +464%, $P < 0.001$) (Fig. 2D–F). Despite the increased retention of ^{14}C-label in BAT, the selectivity of uptake of ^{3}H-label over ^{14}C-label was retained, especially for the 45 and 80 nm-sized particles. In fact, the majority of [^{3}H]TO-depleted [^{14}C]CO-containing core remnants were still taken up by the liver (Fig. 2).

In contrast to the high uptake of [^{3}H]TO-derived activity by BAT, at 21°C and 7°C, the uptake of [^{14}C]CO by BAT was low for both the 45 nm particles (2%/g and 6%/g, respectively) and the 80 nm particles (3%/g and 7%/g, respectively) (Fig. 2), pointing to lipolysis-mediated FA uptake by BAT with minimal remnant particle retention. Uptake of [^{14}C]CO in BAT was markedly higher for the 150 nm particles, especially after cold exposure, suggesting that whole particle uptake may be more relevant for larger TRLs and is further stimulated by cold.

As a measure for the selective retention of FA versus core remnants, we calculated the lipolysis index for all organs as the ratio of ^{3}H-activity (%/g) and ^{14}C-activity (%/g) (**Fig. 3**). For particles of 45, 80, and 150 nm, the lipolysis index of the liver was far below 1, confirming the primary role of the liver in the uptake of TG-poor core remnants. Despite that the uptake by the spleen increased with particle size, the lipolysis index approximated 1 for all particle sizes, consistent with uptake of whole particles through phagocytosis (12, 13). For all particles sizes, the lipolysis index of classical organs involved in the lipolysis-mediated uptake of lipoprotein TG-derived FA (i.e., skeletal muscle, WAT) exceeded 1, representing lipolysis-mediated uptake of FA over CO. In analogy, BAT showed a similarly high lipolysis index (>1) for particles of all sizes. The lipolysis index of BAT for the 150 nm-sized particles was lower than that of the 45 nm and 80 nm-sized particles, and this was also found for skeletal muscle and WAT. The

TABLE 1. List of primer sequences of qRT-PCR

Gene	Forward Primer	Reverse Primer
36b4	GGACCCGAGAAGACCTCCTT	GCACATCACTCAGAATTTCAATGG
β2-microglobulin	TGACCGGCTTGTATGCTATC	CAGTGTGAGCCAGGATATAG
Cd36	GCAAAGAACAGCAGCAAAATC	CAGTGAAGGCTCAAAGATGG
Lpl	CCCTAAGGACCCCTGAAGAC	GGCCCGATACAACCAGTCTA

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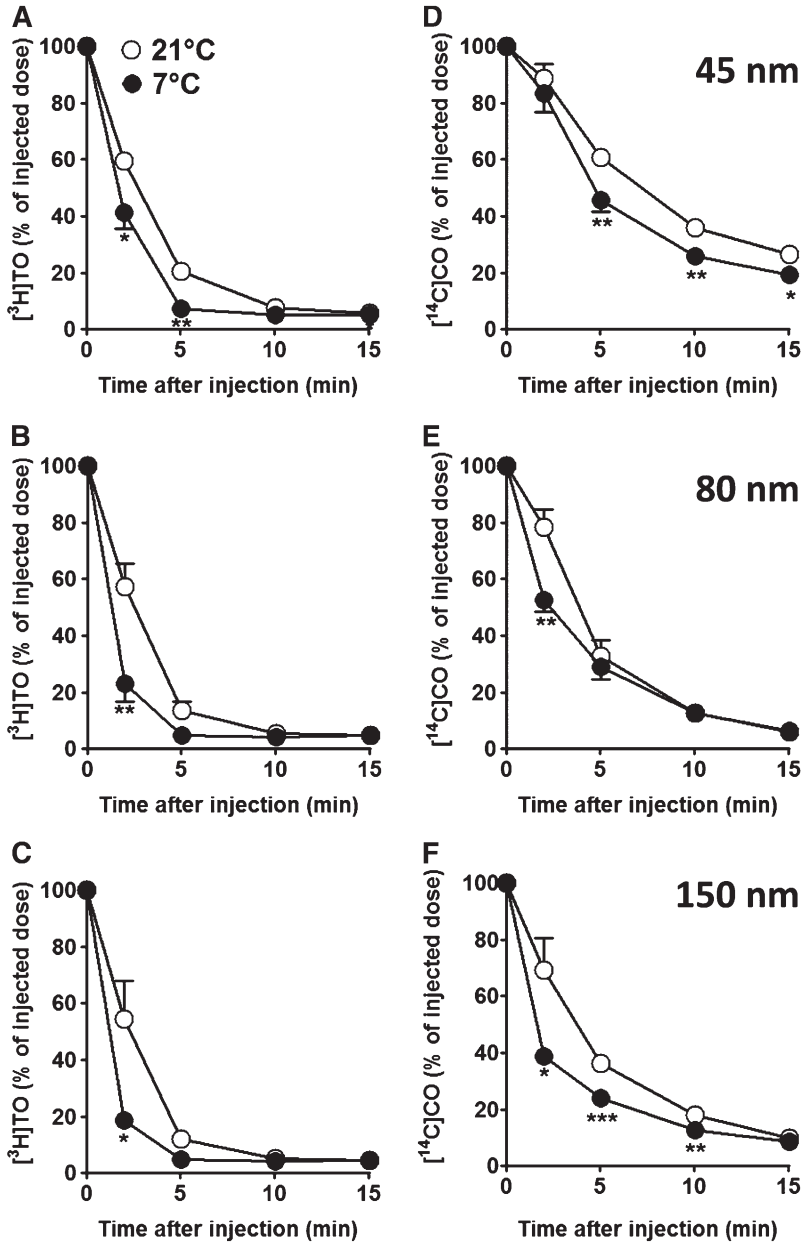

Fig. 1. Cold exposure enhances plasma clearance of double-labeled TRL-mimicking particles. [^{3}H]TO- and [^{14}C]CO-labeled TRL-mimicking particles of different sizes (45, 80, and 150 nm) were injected intravenously into 4 h fasted mice that had been exposed to an ambient temperature of 21°C (open symbols) or 7°C (closed symbols) for 24 h prior to the experiment. Blood was collected at the indicated time points, and [^{3}H]TO activity (A–C) as well as [^{14}C]CO activity (D–F) were measured in plasma. Values are means ± SEM (n = 6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the 21°C group.

lipolysis index in BAT was low (2 ± 0.14) only for 150 nm-sized particles at 7°C indicating that substantial whole particle uptake does take place by BAT upon cold exposure. Thus, these data suggest that TG-derived FA from different-sized TRLs (ranging from small VLDL to chylomicrons) are taken up by skeletal muscle, WAT, and BAT mainly by lipolysis-mediated FA uptake rather than whole particle uptake.

Retention of core remnants in BAT is due to partial uptake

Next, we assessed whether the relatively high retention of [^{14}C]CO from 150 nm particles in BAT was due to uptake by cells within BAT (e.g., whole particle uptake) or may represent retention in the capillaries. To this end, we prepared 150 nm particles containing [^{3}H]COE, which in contrast to [^{14}C]CO cannot be metabolized intracellularly by lysosomal acid lipase and thus remains in the cell. We hypothesized that actual uptake of whole particles would result in long-term retention of [^{3}H]COE activity in BAT, while mere retention of particles in the capillaries within BAT would result in lowering of BAT-associated radiolabel in time.

Consistent with our previous results, cold exposure accelerated the clearance of 150 nm-sized [^{3}H]COE particles from plasma (21°C: $t_{1/2}$ = 5.5 min vs. 28°C: $t_{1/2}$ = 3.3 min, $P < 0.01$). Furthermore, at 21°C, the liver was the largest contributor to uptake of [^{3}H]COE after 15 min (48%/g), 60 min (52%/g), and 300 min (55%/g), and uptake by BAT was low for all time points (7, 7, and 4%/g, respectively) (**Fig. 4A**). At 7°C, the uptake of [^{3}H]COE by the liver at the various time points was comparable to 21°C (Fig. 4B), while uptake by BAT was higher at 7°C (18, 16, and 13%/g after 15, 60, and 300 min, respectively) compared with 21°C. Interestingly, after cold exposure the uptake of [^{3}H]COE by BAT is comparable to the uptake of

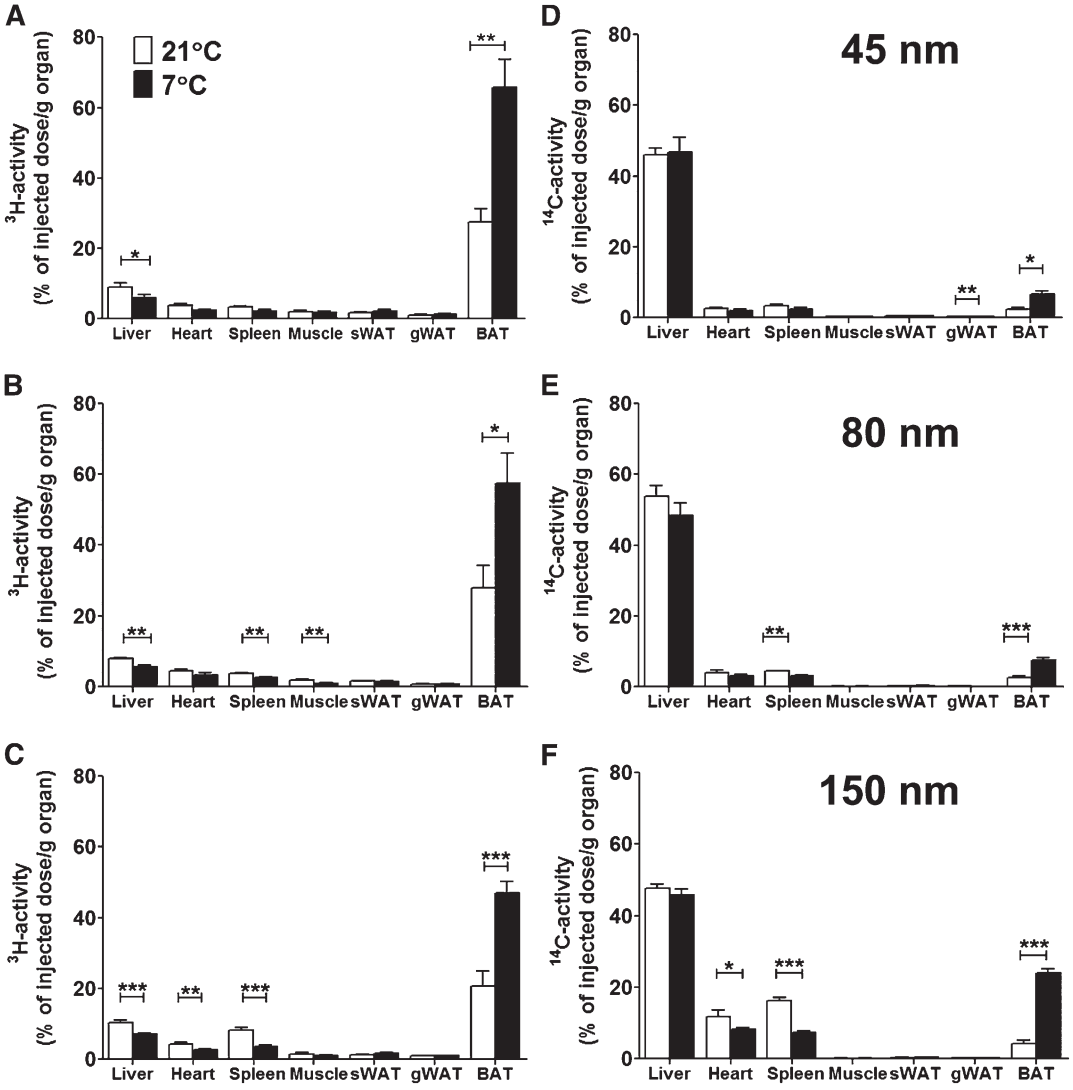

Fig. 2. Cold exposure increases uptake of double-labeled TRL-mimicking particles by BAT. [^{3}H]TO- and [^{14}C]CO-labeled TRL-mimicking particles of different sizes (45, 80, and 150 nm) were injected intravenously into 4 h fasted mice that had been exposed to an ambient temperature of 21°C (open symbols) or 7°C (closed symbols) for 24 h prior to the experiment (see Fig. 1). Uptake of [^{3}H]TO-derived activity (A–C) and [^{14}C]CO activity (D–F) was determined in various organs and expressed as percentage of the injected dose per gram wet tissue weight. Values are means ± SEM (n = 6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the 21°C group. gWAT, gonadal WAT; sWAT, subcutaneous WAT.

[^{14}C]CO from the 150 nm particles, indicating that the potential intracellular metabolism of [^{14}C]CO by lysosomal acid lipase does not seem to be an important factor in our data. The observation that retention of [^{3}H]COE was still found after 300 min, both at 21°C and 7°C, suggests that whole particle uptake by BAT takes place at least to some extent.

Exposure to thermoneutral temperature lowers selective FA uptake from TRL-mimicking particles by BAT

To investigate the mode of TRL-derived FA uptake by BAT under conditions of reduced sympathetic input toward BAT (2), we next performed a clearance experiment with the [^{3}H]TO and [^{14}C]CO double-labeled TRL-mimicking particles of all sizes in mice that were exposed to thermoneutrality (28°C) versus normal room temperature (21°C) for 4 h prior to the experiment. For particles of 45, 80, and 150 nm, plasma [^{3}H]TO clearance was attenuated at 28°C (**Fig. 5A–C**), mainly due to lower uptake of ^{3}H-activity by BAT (−66%, $P < 0.01$; −74%, $P < 0.01$; and −76%, $P < 0.001$, respectively) (**Fig. 6A–C**). Accordingly, 4 h of thermoneutrality increased plasma TG levels by a marked 88% (28°C: 0.79 ± 0.04 vs. 21°C: 0.42 ± 0.02 mM, $P < 0.001$). Again, plasma [^{14}C]CO clearance was slower for all particle sizes as compared with [^{3}H]TO clearance (Fig. 5D, E), and the liver was the main contributor of [^{14}C]CO uptake (Fig. 6D–F). Thermoneutrality had an opposite effect on [^{14}C]CO uptake by BAT as compared with cold exposure. Although, the lipolysis index was lower for 150 nm-sized particles at thermoneutrality, more importantly, the index was for all sized particles larger than 1 (**Fig. 7**). Thus, these data demonstrate that at thermoneutral temperature, FAs from different-sized TRL-mimicking particles are mainly taken up through selective FA uptake by BAT.

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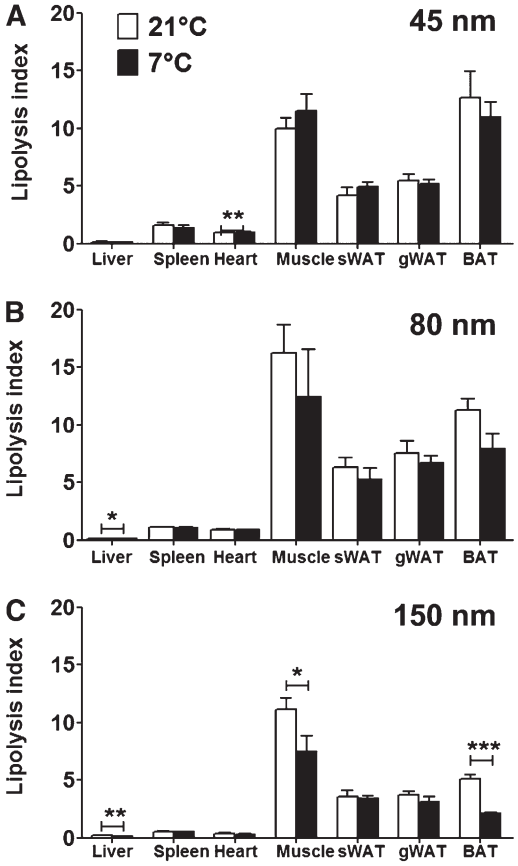

Fig. 3. Lipolysis index in muscle, WAT, and BAT points to selective FA uptake at 21°C and 7°C. [^{3}H]TO- and [^{14}C]CO-labeled TRL-mimicking particles of different sizes (45, 80, and 150 nm) were injected intravenously into 4 h fasted mice that had been exposed to an ambient temperature of 21°C (open symbols) or 7°C (closed symbols) for 24 h prior to the experiment (see Fig. 1). From the uptake of [^{3}H]TO-derived activity and [^{14}C]CO activity by the various organs (see Fig. 2), the lipolysis index was calculated as the ratio of ^{3}H-activity (%/g) and ^{14}C-activity (%/g) for particles of 45 nm (A), 80 nm (B), and 150 nm (C). Values are means ± SEM (n = 6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the 21°C group.

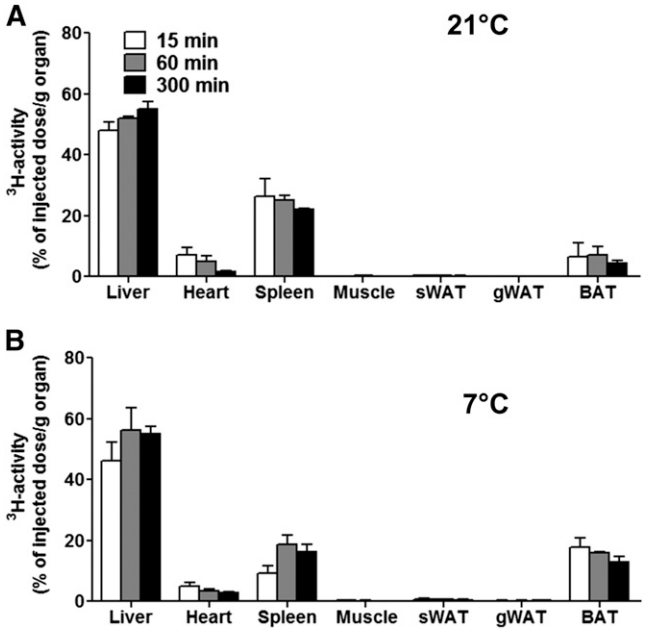

Fig. 4. Retention of core remnants within BAT is at least partly due to uptake by BAT. [^{3}H]COE-labeled TRL-mimicking particles of 150 nm were injected intravenously into 4 h fasted mice that had been exposed to an ambient temperature of 21°C (A) or 7°C (B) for 24 h. Uptake of [^{3}H]COE-derived activity was determined after 15, 60, and 300 min in various organs, and expressed as percentage of the injected dose per gram wet tissue weight. Values are means ± SEM (n = 6).

DISCUSSION

BAT has recently been identified as a major player in TG metabolism (5), but the mechanism by which BAT takes up FA from TG-rich VLDL and chylomicrons had not been fully established yet. In the present study, by performing kinetic studies with both [^{3}H]TO and [^{14}C]CO double-labeled TRL-mimicking particles of different sizes (ranging from small VLDL to chylomicrons) and [^{3}H]COE-labeled TRL-mimicking particles of 150 nm, we provide evidence that BAT, independent of particle size or environmental temperature, mainly takes up FA after lipolysis-mediated liberation from TRL-mimicking particles. Uptake of core remnants or whole particles by BAT does take place to some extent, especially for larger particles and at lower environmental temperature.

LPL, expressed on endothelial cells in the heart, muscle, and WAT, but also in BAT, is crucially involved in hydrolysis of TRLs, resulting in release of FA and subsequent uptake by the adjacent tissue (14). Cellular uptake of FA is mediated by various cell surface receptors, including FA transport proteins and CD36 (15). BAT activation has repeatedly been shown to result in enhancement of LPL activity as well as in increased *Cd36* expression (5, 6). In fact, the LPL/CD36 route is required for TRL-derived FA uptake by BAT as inhibition of local LPL activity in BAT abolished uptake of [^{3}H]oleate and *Cd36*$^{-/-}$ mice show cold intolerance due to inability to take up FA by BAT (5). Therefore, it would make physiological sense if in BAT, as in muscle and WAT, VLDL and chylomicron-derived TGs are primarily taken up after LPL-mediated delipidation of the particle, resulting in the generation of TG-poor remnant particles that can subsequently been taken up by the liver.

The importance of delipidation of particles by BAT is supported by the data of the present study, which show high uptake of [^{3}H]TO-derived activity and relatively low [^{14}C]CO uptake in BAT following injection of differently sized TRL-mimicking particles. Accordingly, lipolysis indices in BAT were high as compared with liver and comparable to those found in WAT and muscle, indicating that BAT is in fact as efficient in delipidating TRLs as WAT and muscle. As these results were found at thermoneutrality, room temperature, and upon cold exposure, it is suggested that the mode of TRL-derived FA uptake is largely independent of BAT activation status. Accordingly, a study by Laplante et al. (6) showed that BAT activation by means of PPARγ agonism results in enhanced uptake of [^{3}H]oleate from VLDL-like emulsion particles. However, as

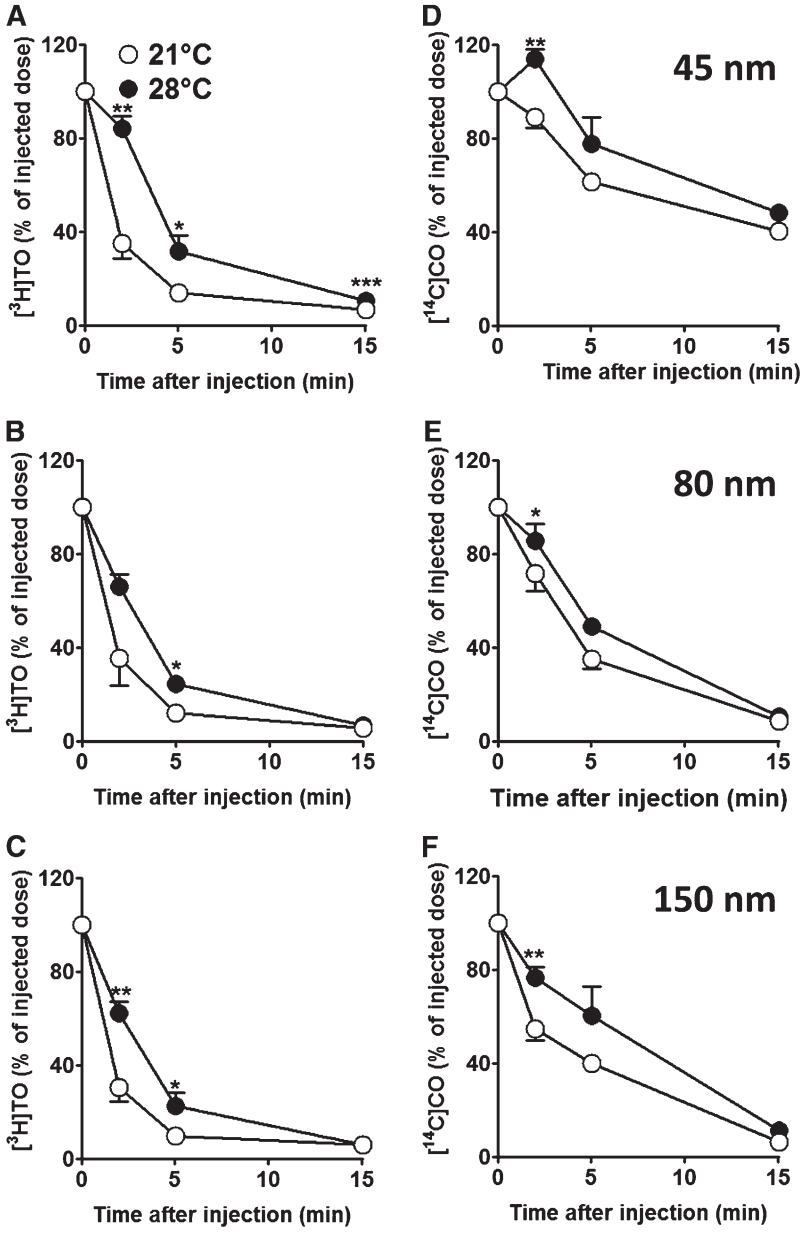

Fig. 5. Thermoneutrality attenuates plasma clearance of double-labeled TRL-mimicking particles. [^{3}H]TO- and [^{14}C]CO-labeled TRL-mimicking particles of different size (45, 80, and 150 nm) were injected intravenously into 4 h fasted mice that had been exposed to an ambient temperature of 21°C (open symbols) or 28°C (closed symbols) for 4 h prior to the experiment. Blood was collected at the indicated time points, and [^{3}H]TO activity (A–C) and [^{14}C]CO activity (D–F) were measured in plasma. Values are means ± SEM (n = 6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the 21°C group.

no nonreleasable core label was used in their study, no definitive statements could be made on whether the uptake truly represented selective FA uptake. Interestingly, the [^{3}H]oleate uptake in BAT highly correlated with the enhanced LPL activity that occurred in the tissue, suggesting that clearance of VLDL-TG and tissue uptake of the radiolabeled FA was mainly determined by LPL-mediated hydrolysis of TG.

Furthermore, upon cold exposure we found somewhat higher ^{14}C-activity in BAT after injection of the 150 nm-sized TRL-mimicking particles as compared with the smaller (45 and 80 nm) VLDL-like particles. This is in line with the previous study of Bartelt et al. (5) in which retention of chylomicron-sized particles (~250 nm) was found in BAT. To determine whether chylomicron-sized particles are taken up by BAT or only show retention in capillaries in BAT during lipolysis, we determined the retention of [^{3}H]COE-labeled 150 nm-sized particles within BAT in time. We showed that both at 21°C and 7°C [^{3}H]COE-derived activity was still present in BAT after 300 min, suggesting that retention of remnants in BAT is at least partly due to cellular uptake. The remnants that are released from the capillaries within BAT in time are most likely eventually taken up by the liver.

We should point out that we used TRL-mimicking particles as models for TG-rich lipoproteins. We previously showed that these emulsion particles rapidly acquire an array of exchangeable apos from serum, including apoE, apoCs, apoAIV, apoAI, apoAII, and apoD (9, 10), and that the hepatic uptake of their core remnants is blocked by lactoferrin (10), which is consistent with apoE-mediated hepatic uptake. In fact, the in vivo kinetics, in rats, of 150 nm-sized [^{3}H]CO-labeled emulsion particles (models for small chylomicrons) (10) are very similar to those of [^{3}H] vitamin A-labeled native chylomicrons (16), with similar clearance rate from plasma ($t_{1/2}$ ~2 min) and uptake by

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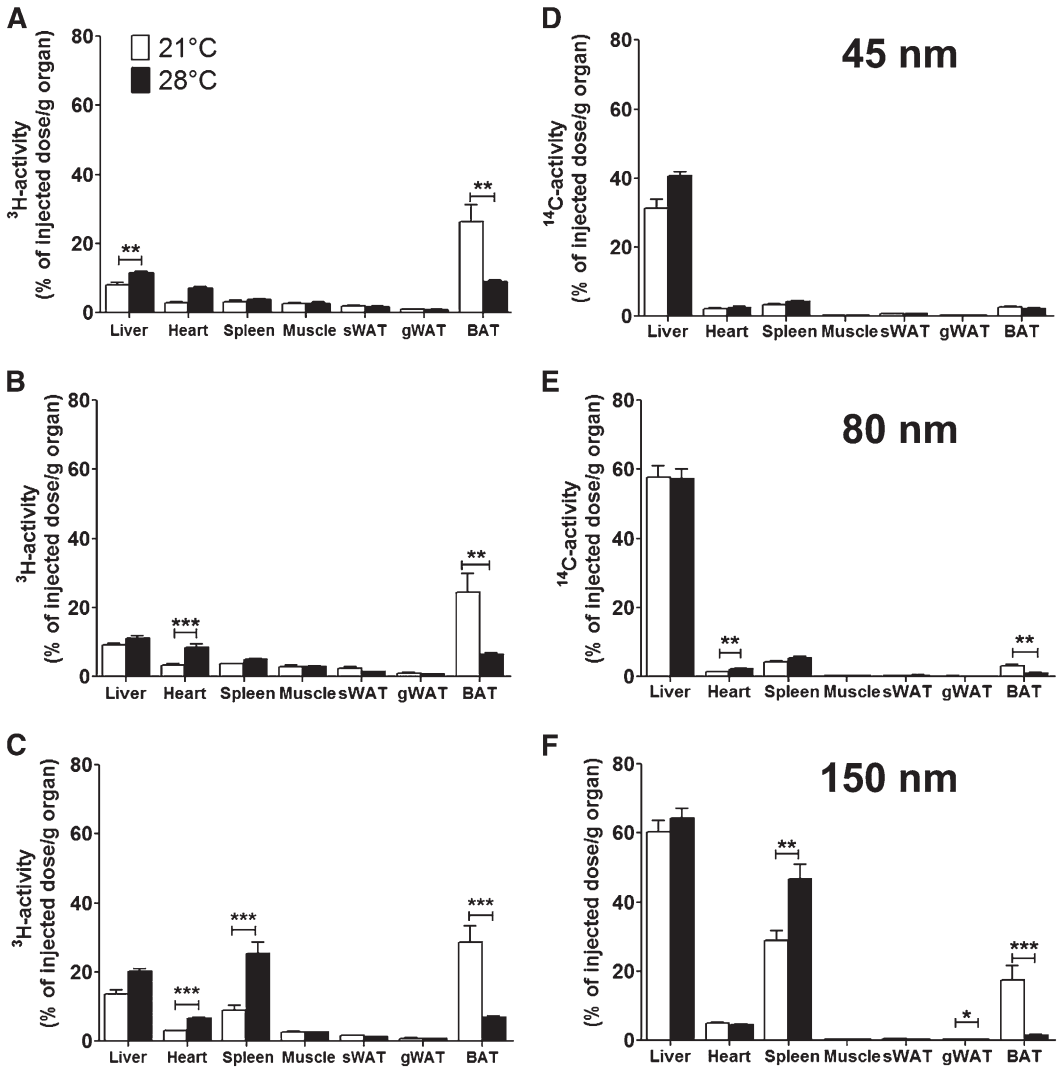

Fig. 6. Thermoneutrality attenuates uptake of double-labeled TRL-mimicking particles by BAT. [^{3}H]TO- and [^{14}C]CO-labeled TRL-mimicking particles of different size (45, 80, and 150 nm) were injected intravenously into 4 h fasted mice that had been exposed to an ambient temperature of 21°C (open symbols) or 28°C (closed symbols) for 4 h prior to the experiment (see Fig. 5). Uptake of [^{3}H]TO- (A–C) and [^{14}C]CO-derived radioactivity (D–F) was determined in various organs, and expressed as percentage of the injected dose per gram wet tissue weight (D–F). Values are means ± SEM (n = 6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the 21°C group.

the liver (~65–75% after 30 min). Lactoferrin reduced the uptake by the liver of both emulsion particles and chylomicrons by ~75% (16), and liver cell distribution studies confirmed that hepatocytes accounted for ~75% of the total uptake by the liver (16). Taken together, it is likely that the present findings for TRL-mimicking emulsion particles can be translated to endogenous TLRs. We demonstrated that cold exposure for 24 h results in a massive 43% reduction of plasma TG levels in mice. These data are in full accordance with a previous study of Bartelt et al. (5) where cold exposure resulted in normalization of plasma TG levels in hyperlipidemic mice. Altogether, these data suggest that cold exposure can be used as therapeutic tool to combat hypertriglyceridemia. Indeed, the findings that metabolically active BAT stores exist in adult humans (17–19) and that BAT volume and activity are lower in obese subjects (18) have increased interest in the therapeutic potential of BAT to combat obesity and related disorders, such as dyslipidemia. Also in humans, BAT likely substantially contributes to TG metabolism. In a recent study by Ouellet et al. (20), human subjects were cooled for 2 h followed by infusion of the FA-tracer ^{18}F-fluoro-thiaheptadecanoic acid and performance of a positron-emission tomography/computerized tomography scan. Indeed, cold exposure resulted in enhanced FA uptake by BAT as compared with muscle and WAT. It is likely that human BAT also utilizes FA from circulating lipoproteins, though this has not been investigated yet.

In conclusion, we show that BAT takes up TRL-derived TG mostly after lipolysis, and this is consistent for TRLs ranging from small VLDL to chylomicrons. Uptake of whole TRL particles or remnant core particles does also occur, albeit to a low extent, and is higher for

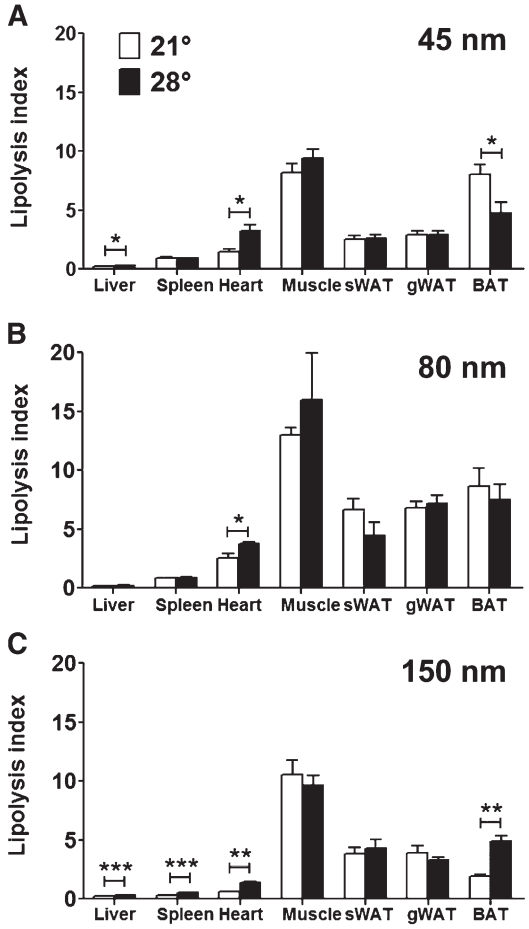

Fig. 7. Lipolysis index in muscle, WAT, and BAT points to selective FA uptake at thermoneutrality. [^{3}H]TO- and [^{14}C]CO-labeled TRL-mimicking particles of different sizes (45, 80, and 150 nm) were injected intravenously into 4 h fasted mice that had been exposed to an ambient temperature of 21°C (open symbols) or 28°C (closed symbols) for 4 h prior to the experiment (see Fig. 5). From the uptake of [^{3}H]TO- and [^{14}C]CO-derived radioactivity in the various organs (see Fig. 6), the lipolysis index was calculated as the ratio of ^{3}H-activity (%/g) and ^{14}C-activity (%/g) for particles of 45 nm (A), 80 nm (B), and 150 nm (C). Values are means ± SEM (n = 6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared with the 21°C group.

larger particles and upon cold exposure. Future studies should elucidate the exact uptake mechanisms in addition to the mechanism by which FAs are taken up by human BAT.

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