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Brown adipose tissue takes up plasma triglycerides mostly after lipolysis. BAT takes up TG-derived fatty acids mostly after lipolysis.
Chapter 5

Abstract

Brown adipose tissue (BAT) produces heat by burning TG 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 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 CE. At thermonutrality (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.

Key terms: • Adipose tissue • Cholesterol • Chylomicrons • Lipoproteins/metabolism • Lipids • Lipoprotein lipase • Lipolysis and fatty acid metabolism • Triglycerides
Selective FA uptake by BAT

Abbreviations

\[^{3}H\]COE \(^{3}H\)cholesteryl oleoyl ether
\[^{3}H\]TO \(^{3}H\)glycerol tri\(^{3}H\)oleate ([\(^{3}H\)triolein)
\[^{14}C\]CO \(^{14}C\)cholesteryl oleate
ATGL adipose triglyceride lipase
BAT brown adipose tissue
HSL hormone sensitive lipase
LDLR low-density lipoprotein receptor
MGL monoglyceride lipase
TRL triglyceride-rich lipoprotein
UCP1 uncoupling protein 1
\((g,s)\)WAT (gonadal, subcutaneous) white adipose tissue
**Introduction**

Brown adipose tissue (BAT) is an important player in energy homeostasis due to its ability to combust energy towards heat by virtue of the presence of uncoupling protein 1 (UCP1), a process called non-shivering thermogenesis (1). The most well-known trigger for activation of BAT is cold, which increases sympathetic outflow from the hypothalamic temperature center towards 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 (HSL) and monoglyceride lipase (MGL), resulting in release of FA from TG-filled lipid droplets (3). FA 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, FA 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 non-shivering 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 TG-rich lipoprotein (TRL)-derived FA from the 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[^H]oleate-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 (approx. 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 particle 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 (LDLR) (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
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of FA into BAT by injecting glycerol tri[^3H]oleate and cholesteryl[^14C]oleate 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°C, 21°C and 28°C).

**Materials and Methods**

**Animals and diet**

For all studies 8-10 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 one 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 glycerol tri[^3H]oleate and cholesteryl[^14C]oleate-labeled TRL-mimicking particles of different size (average 45, 80 or 150 nm, n=6 per group). An additional set of mice received (non-degradable)[^3H]cholesteryl oleoyl ether ([^3H]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 glycerol tri-[^3]Holeate ([^3]HTO) (100 µCi) and [^14]C-cholesteryl oleate ([^14]C CO) (10 µCi) (9, 10). In addition, TRL-mimicking particles were prepared with the non-degradable label [^3]H cholesteryl oleoyl ether ([^3]HCOE) (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 under 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]HTO and [^14]C CO, or [^3]HCOE. Plasma volumes were calculated as 0.04706 × body weight (g) (11). After taking the last blood sample, mice were sacrificed by cervical dislocation and perfused with ice-cold PBS containing 10 U/mL heparin via the heart to remove blood and non-internalized TRL-mimicking particles from the organs. Subsequently, the liver, heart, spleen, hindlimb muscle, gonadal white adipose tissue (gWAT), subcutaneous WAT (sWAT) and interscapular brown adipose tissue (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]HTO-, [^14]C CO- and [^3]HCOE-derived radioactivity by the organs was expressed per gram wet tissue weight.
RNA purification and qRT-PCR

RNA was extracted from snap-frozen mouse tissues (approx. 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 qRT-PCR according to the manufacturer’s instructions to produce cDNA. mRNA expression was normalized to \( \beta_2\)-microglobulin and 36b4 mRNA content and expressed as fold change compared to control mice using the \( \Delta\Delta CT \) method. The primers sequences used are listed in Table 1.

Table 1. List of primer sequences of qRT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>36b4</td>
<td>GGACCCGAGAAGACCTCCTT</td>
<td>GCACATCCTCAGAATTTCATGG</td>
</tr>
<tr>
<td>( \beta_2)-microglobulin</td>
<td>TGACCGCCTGTATGCTATC</td>
<td>CAGTGAGCCAGGATATAG</td>
</tr>
<tr>
<td>Cd36</td>
<td>GCAAAGAACACAGCAAAATC</td>
<td>CAGTGAGGCTCAAAGATGG</td>
</tr>
<tr>
<td>Lpl</td>
<td>CCCTAAGGGACCCCTGAAGAC</td>
<td>GGCCCCGATAAACAGTCTA</td>
</tr>
</tbody>
</table>

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, United States). Differences at probability values less than 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, 2.1, 1.6 and 1.7 min) (Figure 1A-C) than that of \(^{14}\)C]CO (t, 4.2, 2.1, and 2.9 min) (Figure 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 (CE), 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.
Figure 1. Cold exposure enhances plasma clearance of double-labeled TRL-mimicking particles. Glycerol tri[3H]oleate ([3H]TO) and [14C]cholesteryl oleate ([14C]CO) labeled triglyceride-rich lipoprotein (TRL)-mimicking particles of different size (45, 80 and 150 nm) were injected intravenously into 4-hour 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 [3H]TO activity (A-C) as well as [14C]CO activity (D-F) were measured in plasma. Values are means ± SEM (n=6) *P<0.05, **P<0.01, ***P<0.001 compared to the 21°C group.

Indeed, in metabolic tissues that express LPL (i.e., heart, muscle, WAT and BAT) the uptake of 3H-activity (Figure 2A-C) was higher than that of 14C-activity (Figure 2D-F), whereas in the liver the uptake of 14C-activity was much higher than that of 3H-activity. Remarkably, the specific uptake of 3H-activity by BAT exceeded that of all other organs, and was much higher (28, 28, and 20% of injected dose/g) (Figure 2A-C) than the uptake of 14C-activity (2, 3
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and 4% of injected dose/g) (Figure 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 [3H]TO for particles of 45, 80 and 150 nm as compared to 21°C (t_1/2 = 1.3, 1.5 and 1.6 min, respectively) (Figure 1A-C), due to a lipolysis-mediated enhanced uptake of [3H]-activity by BAT (+139%, P<0.01; +111%, P<0.05, and +150%, P<0.001) (Figure 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 [14C]CO (Figure 1D-F), which was accompanied by an increased retention of [14C]-label by BAT (+168%, P<0.05; +181%, P<0.001, and +464%, P<0.001) (Figure 2D-F). Despite the increased retention of [14C]-label in BAT, the selectivity of uptake of [3H]-label over [14C]-label was retained, especially for the 45 and 80 nm sized particles. In fact, the majority of [3H]-depleted [14C]CO-containing core remnants were still taken up by the liver (Figure 2).

In contrast to the high uptake of [3H]TO-derived activity by BAT, at 21°C and 7°C, the uptake of [14C]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) (Figure 2), pointing to lipolysis-mediated FA uptake by BAT with minimal remnant particle retention. Uptake of [14C]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 TRL and is further stimulated by cold.

As a measure for the selective retention of FA vs core remnants, we calculated the lipolysis index for all organs as the ratio of [3H] activity (%/g) and [14C]-activity (%/g) (Figure 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 particle 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 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.
Cold exposure increases uptake of double-labeled TRL-mimicking particles by BAT. [3H]TO and [14C] CO labeled TRL-mimicking particles of different size (45, 80 and 150 nm) were injected intravenously into 4-hour 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 [3H] TO-derived activity (A-C) and [14C]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 to the 21°C group.
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Figure 3. Lipolysis index in muscle, WAT and BAT points to selective FA uptake at 21°C and 7°C. [3H] TO and [14C] CO labeled TRL-mimicking particles of different size (45, 80 and 150 nm) were injected intravenously into 4-hour 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 [3H]TO-derived activity and [14C]CO activity by the various organs (see Fig 2) the lipolysis index was calculated as the ratio of [3H]-activity (%/g) and [14C]-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 to the 21°C group.

Retention of core remnants in BAT is due to partial uptake

Next we assessed whether the relatively high retention of [14C]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 [3H]-labeled cholesteryl oleoyl ether ([3H]COE), which in contrast to [14C]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 [3H]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 [3H]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 [3H]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). (Figure 4A). At 7°C, the uptake of [3H]COE by the liver at the various time points was comparable to 21°C (Figure 4B), while uptake by BAT was higher at 7°C (18, 16 and 13%/g after 15, 60 and 300 minutes, respectively) compared to 21°C. Interestingly, after cold exposure the uptake of [3H]COE by BAT is comparable to the uptake of [14C]CO from the 150 nm particles, indicating that the potential intracellular metabolism of [14C]CO by lysosomal acid lipase does not seem to be an important factor in our data. The observation that retention of [3H]COE was still found after 300 minutes, both at 21°C and 7°C, suggests that whole particle uptake by BAT takes place at least to some extent.

![Figure 4](image_url)

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

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 towards 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) vs 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 (Figure 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) (Figure 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 to [\(^{3}\)H]TO clearance (Figure 5D-E) and the liver was the main contributor of [\(^{14}\)C]CO uptake (Figure 6D-F). Thermoneutrality had an opposite effect on [\(^{14}\)C]CO uptake by BAT as compared to 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 (Figure 7). Thus, these data demonstrate that at thermoneutral temperature, FA from different sized TRL-mimicking particles are mainly taken up through selective FA uptake by BAT.
Figure 5. Thermoneutrality attenuates plasma clearance of double-labeled TRL-mimicking particles. Glycerol tri[3H]oleate ([3H]TO) and [14C]cholesterol oleate ([14C]CO) labeled triglyceride-rich lipoprotein (TRL)-mimicking particles of different size (45, 80 and 150 nm) were injected intravenously into 4-hour 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 [3H]TO activity (A-C) and [14C]CO activity (D-F) were measured in plasma. Values are means ± SEM (n=6) *P<0.05, **P<0.01, ***P<0.001 compared to the 21°C group.
Figure 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-hour 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 to the 21°C group.
Figure 7. Lipolysis index in muscle, WAT and BAT points to selective FA uptake at thermoneutrality. [\textsuperscript{3}H]TO and [\textsuperscript{14}C]CO labeled TRL-mimicking particles of different size (45, 80 and 150 nm) were injected intravenously into 4-hour 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 [\textsuperscript{3}H]TO- and [\textsuperscript{14}C]CO-derived radioactivity in the various organs (see Fig. 6), the lipolysis index was calculated as the ratio of \textsuperscript{3}H-activity (%/g) and \textsuperscript{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 to the 21°C group.
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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 size (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.

Lipoprotein lipase (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 TG are primarily taken up after LPL-mediated delipidation of the particle, resulting in the generation of TG-poor remnant particles that can subsequently be 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 to 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 both 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 PPAR\(\gamma\) agonism results in enhanced uptake of \[^{3}H\]oleate from VLDL-like emulsion particles. However, as no non-releasable 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 to 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 (approx. 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 [%H]COE-labeled 150 nm-sized particles within BAT in time. We showed that both at 21°C and 7°C [%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 apolipoproteins 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 [%H]CO-labeled emulsion particles (models for small chylomicrons) (10) are very similar to those of [%H]vitamin A-labeled native chylomicrons (16), with similar clearance rate from plasma (t½ approx. 2 min) and uptake by the liver (approx. 65-75% after 30 min). Lactoferrin reduced the uptake by the liver of both emulsion particles and chylomicrons by approx. 75% (16), and liver cell distribution studies confirmed that hepatocytes accounted for approx. 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 18F-fluoro-thiaheptadecanoic acid (18F-FTHA) and performance of a PET-CT scan. Indeed, cold exposure resulted in enhanced FA uptake by BAT as compared to 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 larger particles and upon cold exposure. Future studies should elucidate the exact uptake mechanisms in addition to the mechanism by which FA are taken up by human BAT.
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Chapter 5

References


Selective FA uptake by BAT


