Fatty acid cycling in the fasting rat

BELLA KALDERON, NINA MAYOREK, ELLIOT BERRY, NOAM ZEVIT, AND JACOB BAR-TANA
Department of Human Nutrition and Metabolism, Faculty of Medicine, Hebrew University, Jerusalem 91120, Israel

Received 1 November 1999; accepted in final form 5 January 2000

Kalderon, Bella, Nina Mayorek, Elliot Berry, Noam Zevit, and Jacob Bar-Tana. Fatty acid cycling in the fasting rat. Am J Physiol Endocrinol Metab 279: E221–E227, 2000.—Adipose tissue lipolysis and fatty acid reesterification by liver and adipose tissue were investigated in rats fasted for 15 h under basal and calorigenic conditions. The fatty acid flux initiated by adipose fat lipolysis in the fasted rat is mostly futile and is characterized by reesterification of 57% of lipolyzed FFA into VLDL-TG (17, 35). These are hydrolyzed by intravascular lipoprotein lipase, thought to be secondarily reesterified in liver into very low density lipoprotein-triglycerides (VLDL-TG). Total body calorigenesis induced by thyroid hormone treatment and liver-specific calorigenesis induced by treatment with β,β′-tetramethylhexadecanedioic acid (Medica 16) are characterized by a 1.7- and 1.3-fold increase in FFA oxidation, respectively, maintained by a 1.5-fold increase in adipose fat lipolysis. Hepatic reesterification of plasma FFA into VLDL-TG is negligible under both calorigenic conditions. Hence, total body fatty acid metabolism is regulated by adipose tissue as both source and sink. The futile nature of fatty acid cycling allows for its fine tuning in response to metabolic demands.

The transition to the fasting state, in which free fatty acids (FFA) derived by adipose tissue lipolysis become a major source of oxidizable metabolic fuel for heart, skeletal muscle, and liver, is accompanied by major changes in overall lipid and fatty acid metabolism. The FFA cycle is initiated by lipolysis of adipose triglycerides (TG) catalyzed by hormone-sensitive lipase (HSL). Fat lipolysis may either yield plasma FFA or result in intracellular reesterification of lipolyzed FFA back into adipose fat before their release (primary reesterification). Plasma FFA that escape direct oxidation are thought to be secondarily reesterified in liver into very low density lipoprotein-triglycerides (VLDL-TG) (17, 41). These are hydrolyzed by intravascular lipoprotein lipase, followed by reuptake of the released FFA into adipose tissue, muscle, or liver. However, secondary reesterification of FFA into VLDL-TG has recently been shown in humans to account for only a minor fraction of total FFA released from adipose tissue, thus leaving unsolved the fate of plasma FFA that escape oxidation or secondary reesterification into VLDL-TG (12).

Long-chain fatty acid metabolism is closely associated with calorigenesis induced by hormones and drugs and is therefore of particular interest under conditions of treatment with thyroid hormones (TH). However, despite studies concerned with the effects exerted by TH on discrete steps of fatty acid metabolism, a quantitative and integrative in vivo view of long-chain fatty acid metabolism in TH-treated animals is still lacking. Thus TH was reported to induce lipolysis in isolated adipose tissues (1) or adipocytes (18, 26) as a result of sensitization of adipocytes to the lipolytic effect of catecholamines (5, 18). Increased lipolysis was corroborated by increased total body lipid oxidation as well as a decrease in adipose tissue weight (31). However, an integrative view consisting of quantitative evaluation of the in vivo production rates of FFA and glycerol as well as the FFA fraction that is either oxidized or secondarily reesterified in rats treated with TH is still lacking. Similarly, despite in vitro evidence suggesting that hepatic VLDL release (11, 39) and lipoprotein lipase activity (10, 34) are affected by TH treatment, the in vivo production rate of hepatic VLDL-TG as a fraction of the overall fatty acid flux still remains to be investigated.

Calorigenesis induced by β,β′-tetramethylhexadecanedioic acid (Medica 16) (2) is apparently similar to that induced by TH. Thus, similarly to TH, Medica 16 treatment results in sensitization of adipose tissue to catecholamines, leading to lipolysis, ketogenesis, and weight reduction (38). Furthermore, similarly to TH, Medica 16 treatment induces a pronounced decrease in liver phosphate and redox potentials (24, 25) with a concomitant decrease in mitochondrial membrane potential observed in both isolated mitochondria and liver cells (19). The thymoimetic activity of Medica 16 is further reflected by its capacity to induce liver genes classically considered to be TH dependent, e.g., malic enzyme, mitochondrial glycerol-3-phosphate dehydro-
genase, S14, and others (20, 21). However, in contrast to TH, the calorigenic activity of Medica 16 is liver specific. Thus, whereas TH induces a decrease in phosphate potential in both liver and heart, the decrease in phosphate potential induced by Medica 16 is observed in liver only (24, 25). Similarly, transcriptional activation by TH of TH-dependent genes is observed in both liver and heart, whereas the respective nuclear activity of Medica 16 is, again, liver specific. Also, the thyrometabolic activity of Medica 16 has been shown to be independent of TH levels and is not transduced by the TH nuclear receptor (20, 21). The apparent liver specificity of Medica 16 has been shown elsewhere to be accounted for by its failure to enter cardiac or skeletal muscle (H. Bdeir, unpublished observations).

Long-chain fatty acid metabolism was evaluated here in the fasting rat in terms of the in vivo fluxes of adipose tissue lipolysis, fatty acid oxidation, and the recycling of fatty acids in liver and adipose tissue as a function of whole body calorigenesis induced by TH or hepatic calorigenesis induced by Medica 16.

METHODS

Animals. Male Albino rats of the Hebrew University strain weighing 300–400 g were fed a standard laboratory chow diet containing 4.5% fat. Rats were treated for 4–6 wk with 390 mg · kg⁻¹ · day⁻¹ Medica 16 by mixing the drug in the powdered diet. Hyperthyroidism was induced by six daily intraperitoneal injections of 10 μg T₃ · 100 g body wt⁻¹ · day⁻¹ in 0.01 M NaOH in saline. Control rats were injected with the vehicle only. All animals had free access to food and drinking water. Animal care and experimental procedures were in accordance with guidelines of the Animal Care Committee of Hebrew University.

Cannulation procedures. Rats fasted for 15 h were restrained in restriction cages (Harvard Apparatus) and cannulated under local anesthesia with lignoamine through the tail artery and vein for blood sampling and isotope infusion, respectively (28). After catheter placement, animals were released to their cages, where they could move freely, and allowed to recover for 90 min. Biting off of tail catheters was prevented by wrapping the tail with a plastic sheath coated with castor oil repellent. Catheter patency was maintained with saline to avoid heparin during measurement of lipolytic rates.

Indirect calorimetry. Whole body O₂ consumption and CO₂ production rates were measured several times before and during the infusion study by open-circuit indirect calorimetry using a NAGA O₂/CO₂ analyzer (Frantzec, Haifa, Israel). The time of urination and urine volumes during the infusion period were recorded using an intracage moisture-sensitive alarm. Urinary nitrogen was determined by Kjeldahl analysis in urine acidified with 1 N H₂SO₄.

FFA, glycerol, and VLDL-TG production rates. Cannulated recovered animals were infused constantly for 150 min (20–30 μl/min) through the tail vein with 98%-enriched [2,2,3H₂]palmitate (Cambridge Isotope Laboratories, Andover, MA) (bound to albumin at a ratio of 6:1) at a rate of 0.16 μmol · min⁻¹ · kg body wt⁻¹ and with 98%-enriched [3H]glycerol (ISOTEC, Miamisburg, OH) in saline at a rate of 0.6 μmol · min⁻¹ · kg body wt⁻¹ using a Harvard Apparatus syringe pump. For measurement of FFA and glycerol production rates, blood samples were withdrawn at intervals of 20–30 min during the last 50–150 min period of constant infusion for measurements of steady-state enrichment of plasma palmitate and glycerol. For measurement of VLDL-TG production rate, the infusion of label was stopped, allowing for a decay in the enrichment of palmitate in plasma VLDL-TG. The enrichment of plasma TG-palmitate was followed for 60–80 min in blood samples collected at intervals of 5–15 min. Sampled blood amounted to 1.5–3.0 ml.

Plasma free and esterified palmitates were extracted with 5 ml of isopropanol-heptane-2 N H₂SO₄ at a ratio of 40:10:1. The upper heptane phase was separated and further extracted with 1 ml of alkaline 70% methanol, followed by extraction of the alkaline methanol with heptane. The heptane phases consisting of plasma TG were combined. The alkaline methanol containing the free fatty salt was then acidified and extracted twice with 2 ml of heptane to recover plasma FFA. The plasma TG and FFA extracts were subjected to further purification by TLC (heptane-diethyl ether-glacial acetic acid at a ratio of 157:39:3.9). The purified fatty acids and TG were derivatized to their methyl esters as described previously (6) and analyzed by gas chromatography-mass spectrometry (GC-MS) as described below.

Plasma glycerol was extracted from 100 μl of plasma by barium hydroxide-zinc sulfate precipitation, and the supernatant was then passed through a mixed cation/anion exchange resin. The eluate was collected in reaction vials and evaporated to dryness. Glycerol was derivatized to its tert-butyldimethylsilyl derivative essentially as described previously (8). Briefly, 50–60 μl of anhydrous acetonitrile were added to dried eluates, followed by the addition of 20 μl of N-methyl-N-(tert-butyldimethylsilyl)trifluoroacetamide. The reaction vials were sealed with Teflon-lined capes, heated in a 110°C heating block for 15 min, and left to cool for another hour at room temperature. The derivatized glycerol was analyzed by GC-MS as described below.

Isotopic enrichment of t-butylidimethylsilyl glycerol and methylpalmitate was determined by GC-MS analysis using a Quatro II Fisons quadrupole mass spectrometer coupled to a gas chromatograph (15m DB-1 GC capillary column; J&W Scientific, Folsom, CA). The mass spectrometer was operated in the electron impact mode at ionization energy of 70 eV and source temperature of 180°C. The mass spectrometer was daily tuned to the 219 and 264 m/e ions of heptacosahexaenoic acid. Methylpalmitate enrichment was determined by selectively monitoring the 270 (M) and 272 m/e (M + 2) ions. The t-butylidimethylsilyl derivative of glycerol was determined by selectively monitoring the 217 (M) and 220 m/e (M + 3) ions. The 217 m/e ion appeared to be the most intense and stable fragment derived from t-butylidimethylsilyl glycerol. Selectively monitoring the ions of 217 and 220 m/e provided an accurate measurement of glycerol enrichment as verified by analysis of glycerol standards of known glycerol enrichments.

Secondary reesterification in adipose tissue. Cannulated recovered animals were primed with 12 μCi [3H]palmitate in saline, followed by constant infusion for 100–280 min with VLDL-TG production rate, the infusion of label was stopped, allowing for a decay in the enrichment of palmitate in plasma VLDL-TG. The enrichment of plasma TG-palmitate was followed for 60–80 min in blood samples collected at intervals of 5–15 min. Sampled blood amounted to 1.5–3.0 ml.

Plasma free palmitate (100 μl) was extracted with isopropanol-heptane-2 N H₂SO₄ at a ratio of 40:10:1, as described above, and derivatized with α-bromoacetophenone (29). The bromophenyl acyl ester was purified by HPLC using a 150 ×
Table 1. Plasma insulin and metabolites in postabsorptive nontreated and TH- and Medica 16-treated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>FFA, mM</th>
<th>TG, mg %</th>
<th>Glucose, mg %</th>
<th>Insulin, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td>1.0 ± 0.1</td>
<td>62 ± 6</td>
<td>72 ± 2</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>TH</td>
<td>1.0 ± 0.1</td>
<td>84 ± 6</td>
<td>89 ± 4</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>Medica 16</td>
<td>0.8 ± 0.1</td>
<td>39 ± 1*</td>
<td>86 ± 4</td>
<td>1.8 ± 0.3</td>
</tr>
</tbody>
</table>

Plasma parameters were determined in rats treated as described in METHODS. Values are means ± SE (n = 4–6 rats). FFA, free fatty acids; TG, triglycerides; TH, thyroid hormones. *Significantly different from respective nontreated animals (P < 0.05).

Table 2. Adipose tissue lipolysis and primary reesterification rates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rn Glycerol, μmol · min⁻¹ · kg⁻¹</th>
<th>Ra FFA, μmol · min⁻¹ · kg⁻¹</th>
<th>Rn FFA/Rn Glycerol</th>
<th>Lipolytic Flux (3Rn Glycerol), μmol · min⁻¹ · kg⁻¹</th>
<th>Primary Reesterification, μmol · min⁻¹ · kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td>16.4 ± 1.0</td>
<td>30.2 ± 0.8</td>
<td>1.84 ± 0.10</td>
<td>49.2 ± 3.0</td>
<td>19.0 ± 2.4</td>
</tr>
<tr>
<td>TH</td>
<td>24.8 ± 1.2*</td>
<td>39.2 ± 0.9*</td>
<td>1.58 ± 0.06*</td>
<td>74.4 ± 3.6*</td>
<td>35.2 ± 3.2*</td>
</tr>
<tr>
<td>Medica 16</td>
<td>23.2 ± 1.1*</td>
<td>33.5 ± 2.7</td>
<td>1.44 ± 0.06*</td>
<td>69.6 ± 3.3*</td>
<td>36.1 ± 2.5*</td>
</tr>
</tbody>
</table>

Total body rates of appearance of glycerol (Rn glycerol) and FFA (Ra FFA) were measured in nontreated and TH- and Medica 16-treated rats as described in METHODS. Primary reesterification was calculated (3Rn glycerol − Ra FFA) as described in METHODS. Values are means ± SE (n = 4–6 rats). *Significantly different from respective nontreated animals (P < 0.05).
lipolytic flux resulted in compromised FFA release into plasma (Rₖ FFA) with a concomitant decrease in the ratio of Rₖ FFA to Rₖ glycerol. Primary reesterification accounted for 39, 47, and 52% of total lipolytic flux in the fasting state in nontreated and TH- and Medica 16-treated rats, respectively.

**Fatty acid oxidation.** Total body O₂ consumption increased by 55% in TH-treated animals compared with age-matched controls (34.2 ± 0.9 vs. 22.1 ± 0.5 ml O₂ · min⁻¹ · kg⁻¹, P < 0.05). Because total body O₂ consumption decreased with age (when normalized to body weight), and in light of the relatively long period of treatment with Medica 16 compared with the short treatment period with TH, O₂ consumption of Medica 16-treated animals was measured twice, following 1 and 6 wk of treatment. O₂ consumption was significantly increased by 15% in Medica 16-treated rats following 1 wk of treatment (25.4 ± 0.6 vs. 22.1 ± 0.5 ml O₂ · min⁻¹ · kg⁻¹, P < 0.05) and persisted through the 6 wk of the treatment period (19.7 ± 0.3 vs. 17.2 ± 0.2 ml O₂ · min⁻¹ · kg⁻¹, P < 0.05).

Total body lipid oxidation rates measured by indirect calorimetry several times during the isotope infusion period were increased 1.7- and 1.3-fold in TH- and Medica 16-treated rats, respectively. The fraction of oxidized FFA out of FFA released from adipose tissue (Rₖ FFA) amounted to 59, 78, and 68% in nontreated, TH-, and Medica 16-treated rats, respectively (Table 3).

**Secondary reesterification.** Secondary reesterification rates were evaluated by subtracting total body FFA oxidation rates from Rₖ FFA. As shown in Table 3, secondary reesterification was significantly decreased in hyperthyroid rats in light of the substantial increase in lipid oxidation.

Hepatic VLDL-TG production was evaluated by analyzing the decay curve of plasma TG-palmitate enrichment when labeled palmitate infusion was interrupted. As shown in Fig. 1, plasma TG-palmitate enrichment reached isotopic steady state after 120–140 min of labeled palmitate infusion compared with 50–60 min for plasma free palmitate. A slow turnover rate for plasma TG-palmitate compared with that for free palmitate was also reflected by the slow decay of plasma TG-palmitate compared with that of free palmitate (Fig. 1). Also, plasma TG-palmitate enrichment in nontreated animals during isotopic steady state amounted to 70 ± 5.5% of plasma free palmitate (Fig. 1), indicating that only ~70% of liver fatty acids incorporated into VLDL-TG were derived in nontreated animals from plasma FFA, whereas 30% were derived from an unlabeled hepatic pool of fatty acids. This nonplasmatic liver pool was significantly decreased in animals treated with TH or Medica 16 and in which plasma TG-palmitate enrichment during isotopic steady state amounted to 82 ± 2.9 and 83 ± 1.8% of plasma free palmitate enrichment, respectively. Hepatic VLDL-TG production rates as calculated from the respective decay of the enrichment curves of the three groups are shown in Table 3. VLDL-TG production accounted for 15% of total body secondary reesterification in nontreated animals, and because only 70% of VLDL-TG were synthesized from plasma FFA, hepatic reesterification accounted for only 9% of secondary reesterification.

### Table 3. FFA oxidation and recycling rates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rᵢ FFA, μmol·min⁻¹·kg⁻¹</th>
<th>FFA Oxidation, μmol·min⁻¹·kg⁻¹</th>
<th>Secondary Reesterification, μmol·min⁻¹·kg⁻¹</th>
<th>Secondary Hepatic Reesterification, μmol·min⁻¹·kg⁻¹</th>
<th>Secondary Adipose Reesterification, μmol·min⁻¹·kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontreated</td>
<td>30.2 ± 0.8</td>
<td>17.9 ± 0.8</td>
<td>12.3 ± 0.9</td>
<td>1.6 ± 0.2</td>
<td>9.0 ± 0.9</td>
</tr>
<tr>
<td>TH</td>
<td>39.2 ± 0.9*</td>
<td>30.6 ± 1.4*</td>
<td>8.6 ± 1.5*</td>
<td>1.2 ± 0.2*</td>
<td>ND†</td>
</tr>
<tr>
<td>Medica 16</td>
<td>33.5 ± 2.7</td>
<td>27.7 ± 0.6*</td>
<td>10.8 ± 0.5</td>
<td>0.5 ± 0.2*</td>
<td>6.5 ± 0.3*</td>
</tr>
</tbody>
</table>

*Rᵢ FFA, total body FFA oxidation, total body reesterification of plasma FFA (secondary reesterification), very low density lipoprotein-TG production rate (secondary hepatic reesterification), and rate of reesterification of plasma FFA into adipose fat (secondary adipose reesterification) were determined in nontreated and TH- and Medica 16-treated rats as described in METHODS. Values are means ± SE (n = 3–6 rats). ND, not determined. *Significantly different from respective nontreated animals (P < 0.05). †FFA incorporation into adipose tissue TG of hyperthyroid animals amounted to 0.145 ± 0.020 μmol·min⁻¹·g adipose tissue⁻¹ (compared with 0.082 ± 0.008 and 0.059 ± 0.03 for nontreated and Medica 16-treated animals, respectively). Assuming total body adipose mass for hyperthyroid animals to be 6.8% of body weight (based on Ref. 31) would result in secondary adipose reesterification of 9.8 ± 1.4 μmol·min⁻¹·kg⁻¹.

*Fig. 1. Plasma free fatty acid (FFA) and triglyceride (TG) enrichment. Plasma free palmitate (●) and TG palmitate (■) enrichment were determined during a period of 145 min of labeled palmitate infusion followed by 80 min of saline infusion. Interruption of [²H₃]palmitate infusion is marked by arrow. Enrichment is expressed in mole percent excess (MPE).*
reesterification or only 3% of total body lipolytic flux. Moreover, Medica 16 and TH treatments resulted in a 3- and 1.5-fold decrease in VLDL-TG production rate, respectively, indicating that hepatic reesterification of FFA under calorigenic conditions accounted for an even lower fraction of total body secondary reesterification. The calorigenesis-induced decrease in hepatic VLDL-TG production was not compromised by changes in liver TG content (data not shown).

The contribution made by adipose tissue to total body secondary reesterification was evaluated by the recovery of labeled palmitate in adipose TG under conditions of isotopic steady state in plasma free palmitate accompanying by linear accumulation of plasma FFA in adipose tissue (Fig. 2). Palmitate incorporation into adipose TG was calculated as the average of adipose tissue labeling in samples taken from the epididymal, perirenal, and subcutaneous fat. No significant differences in palmitate incorporation were observed between different fat pads (data not shown). As shown in Table 3, FFA reesterification in adipose tissue accounted for 60–100% of total body secondary reesterification, indicating that adipose tissue is the primary site for secondary reesterification in rats. Secondary reesterification of FFA in adipose tissue essentially accounted for plasma FFA that escaped oxidation or hepatic reesterification into VLDL-TG under euthyroid or induced calorigenesis. No significant differences were observed in muscle TG content among the various conditions (data not shown).

DISCUSSION

Fatty acid metabolism in fasted rats: an integrative view. Long-chain fatty acid metabolism in the fasting state is initiated by HSL-catalyzed lipolysis of adipose fat, resulting in plasma FFA as a source of metabolic fuel for the heart, skeletal muscle, and liver. Total body FFA oxidation in the nontreated fasted rat amounts to only 36% of total body lipolytic flux, indicating that adipose tissue lipolysis is activated much beyond the requirement for total body fat oxidation. Moreover, plasma FFA channeled into VLDL-TG production is only 5% of Ra FFA, indicating that the oxidizable fatty acid pool in the postabsorptive state consists mainly of plasma FFA released from adipose fat and directly oxidized by respective tissues, rather than fatty acids derived from intravascular lipolysis of VLDL-TG. The higher production rates of VLDL-TG previously reported by Wolfe and Durkot (40) could perhaps result from the use of labeled dog VLDL instead of rat VLDL or from plasma VLDL enrichments lower than isotopic steady-state levels.

Most FFA resulting from fat lipolysis in adipose tissue in the postabsorptive state are reesterified back into TG. Because hepatic reesterification of FFA into VLDL-TG accounts for only a minor fraction of total body FFA flux, most of the reesterified FFA would have to be accounted for by extrahepatic tissues. Indeed, 39% of total body lipolytic FFA was found here to be primarily reesterified into adipose fat even before its release into plasma, in line with a previous report (40) pointing to a ratio of 1:1 for Ra FFA/Ra glycerol in the postabsorptive rat. Another 18% of total body lipolytic FFA was found here to be secondarily reesterified in adipose tissue, making this tissue the most important site of reesterification of recycled FFA in general and of secondary reesterification in particular. Hence, primary and secondary reesterification of lipolytic FFA in adipose tissue accounts for almost all FFA produced by HSL and that escape oxidation in oxidizing tissues. Total body fatty acid metabolism and steady-state levels of plasma FFA appear, therefore, to be controlled by adipose tissue, as both a source and sink.

Similarities and differences between the FFA cycle reported here in fasted rats and previously in fasted humans are worth noting. The overall FFA flux in rats (49.2 ± 3.0 μmol·min⁻¹·kg⁻¹) is ~5- to 15-fold higher than in humans (3–9 μmol·min⁻¹·kg⁻¹) (12, 17, 33). However, primary reesterification in fasted humans is negligible (3, 9, 12, 35; see, however, Refs. 7, 17, 41, and 42), whereas that in fasted rats accounts for 40–50% of the total flux (Table 2; Ref. 39). Because human primary reesterification combined with VLDL-TG production rate may account for a fraction of only 5% of total body FFA turnover in the fasting state (12), and because lipid oxidation accounts for 40% of FFA turnover under these conditions, extrahepatic secondary reesterification must account for at least 50% of human total body FFA turnover. The site(s) for secondary reesterification of FFA in humans remained unresolved (7, 12). However, in light of the general similar-
ities between FFA cycling in human subjects and rats, secondary reesterification is proposed to be essentially carried out by adipose tissue in both species. The implied extensive flux of adipose secondary reesterification of FFA in humans under conditions in which primary reesterification is almost nil may indicate that the primary and secondary reesterification pathways must be limited by distinct steps. Although epididymal, subcutaneous, and perirenal rat adipose tissues were found here to account for similar secondary reesterification rates (based on tissue weight), the specificity of FFA uptake by individual human adipose tissues still remains to be investigated. Lack of FFA uptake by human abdominal subcutaneous adipose tissue in the fasting state as recently claimed by Coppack et al. (9) may indeed indicate that human individual adipose tissues may differ in their FFA extraction capacities.

The fatty acid cycle in TH-treated fasted rats. TH was found here to activate lipolysis of adipose fat 1.5-fold, in line with 3-fold activation previously reported in humans (3). This increase in lipolytic rate may be ascribed to adipose tissue sensitization to catecholamines as a result of the increased expression of β-adrenoreceptors (5, 18). The increase in lipolytic rate by TH was, however, compromised by a dramatic increase in primary reesterification. The increase in lipolytic flux induced by TH was accompanied by a 55% increase in total body O₂ consumption and a 1.7-fold increase in lipid oxidation, in line with a similar increase previously reported by Oppenheimer et al. (31). The increase in oxidation may be ascribed to mitochondrial uncoupling due to transition from a low to a high mitochondrial conductance (23). TH-induced oxidation of FFA limits the extent of secondary reesterification of plasma FFA. The decrease in hepatic VLDL-TG production could perhaps reflect the lower availability of hepatic fatty acids for reesterification under conditions of TH-induced calorigenesis and may account for the somewhat lower plasma TG observed in postabsorptive TH-treated rats. Secondary reesterification in adipose tissue completes the FFA cycle and again accounts for most recycled plasma FFA. In summary, the calorigenic effect of TH is dominated by high turnover of the FFA cycle with a concomitant absolute and relative increase in primary reesterification and FFA oxidation.

The thyromimetic-calorigenic effect of Medica 16. Similarly to TH, Medica 16 was found here to increase adipose fat lipolysis by 41%, in line with activation of lipolysis by Medica 16 in obese, insulin-resistant fa/fa rats (28). Activation of the lipolytic flux under conditions of sensitization to insulin (28) may imply that Medica 16-induced lipolysis of adipose fat is transduced by an insulin-independent pathway. Activation of adipose lipolysis by Medica 16 is indeed accompanied by increased sensitization of adipocytes to variable lipolytic activators (e.g., catecholamines, forskolin, ACTH) (38). The 41% increase in lipolytic flux induced by Medica 16 is only partially compromised by the 27% increase in lipid oxidation, leaving a substantial fraction to primary and secondary reesterification. Similarly to TH, Medica 16 indeed increased primary reesterification of FFA in adipose tissue, in line with that recently reported in obese fa/fa rats, under either basal or hyperinsulinemic-euglycemic clamp conditions (28). Secondary reesterification in liver was, however, significantly inhibited by Medica 16 treatment, leaving most secondary reesterification to adipose tissue. Hence, under conditions of increased total body FFA turnover induced by Medica 16 treatment, FFA reesterification (primary + secondary) in adipose tissue again accounted for 61% of lipolytic flux in the fasting state.

Medica 16 treatment resulted in a 15% increase in total body O₂ consumption with a concomitant 1.3-fold increase in lipid oxidation. Calorigenesis induced by Medica 16 was in line with that in a previous study from this laboratory reporting uncoupling of mitochondrial oxidative phosphorylation by Medica 16 (19). The lower increase in total body O₂ consumption induced by Medica 16 compared with that induced by TH reflects the liver-specific thyromimetic activity of Medica 16 compared with the nonselective calorigenic activity of TH (25).

The pronounced inhibition of hepatic VLDL-TG production by Medica 16 may account for the hypolipidemic effect of Medica 16 in the fasting state. This hypolipidemic effect complements the previously reported hypolipidemic activity of Medica 16 in the absorptive state due to activated clearance of plasma VLDL and chylomicrons as a result of transcriptional suppression of the hepatocyte nuclear factor-4 (HNF-4)-controlled apolipoprotein CIII (14, 15, 22). The postabsorptive hypolipidemic effect of Medica 16 could perhaps reflect transcriptional suppression of other HNF-4-controlled genes involved in liver VLDL assembly and, in particular, of apolipoprotein B and microsomal triglyceride transfer protein. Liver VLDL-TG production could perhaps be further limited by the limited availability of liver FFA for reesterification under conditions of pronounced hepatic calorigenesis induced by Medica 16 treatment, in line with recent reports (27, 36) pointing to the reciprocal interplay between fatty acid oxidation and its hepatic reesterification into VLDL-TG.

In summary, adipose tissue serves as a main source as well as a sink for fatty acid metabolism in the fasted rat. The fatty acid flux initiated in rat adipose tissue is mostly futile and results in reesterification of 57% of lipolyzed FFA back into adipose TG. Two-thirds of FFA reesterification into adipose fat are carried out before FFA release into plasma, whereas the rest results from extraction of plasma FFA. Thirty-six percent of total lipolytic FFA flux is accounted for by net FFA oxidation, whereas only a minor fraction of FFA flux is channeled into hepatic VLDL-TG. Fatty acid cycling in excess of oxidative demands allows for rapid response to sudden changes in energy requirements (41). Moreover, the futile nature of the cycle allows for its fine tuning in response to even mild changes in metabolic demands (30). This high responsiveness of the fatty acid cycle is preserved under conditions of calorigenesis induced by thyroid hormones or Medica 16 due to an increase in lipolytic flux, maintaining essentially constant the oxidative flux-to-cycling flux ratio.
This work was supported by the Israeli Ministry of Science and Technology.

REFERENCES


