Mice Lacking the Thyroid Hormone Receptor-\(\alpha\) Gene Spend More Energy in Thermogenesis, Burn More Fat, and Are Less Sensitive to High-Fat Diet-Induced Obesity

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Unable to activate brown adipose tissue (BAT) thermogenesis, \(\alpha\)T\(_3\)-receptor-deficient mice (Thra-0/0) are cold intolerant. Our objective was to investigate the impact on energy economy and mechanisms of the alternate facultative thermogenesis developed. Energy expenditure (oxygen and food consumption) is elevated in Thra-0/0 mice reared at room temperature. Such difference disappears at thermoneutrality (30°C) and expands as ambient temperature becomes colder (\(P < 0.001\)). Despite eating more, Thra-0/0 are leaner than wild-type (WT) mice (\(P < 0.01\)), whereas these, whether on chow or high-fat diet, gained more weight (g/d: 0.12 ± 0.002 vs. 0.08 ± 0.002 and 0.25 ± 0.005 vs. 0.17 ± 0.005, respectively) and adiposity than Thra-0/0 mice (\(P < 0.001\)). The respiratory quotient was lower in Thra-0/0 than WT mice (\(P < 0.001\)) after feeding or fasted, on chow or high-fat diet, indicating a preference for fat as fuel, which was associated with increased lipoprotein lipase (LPL) expression in skeletal muscle of Thra-0/0 mice but with no differences in gene expression in white adipose tissue. Type-2 deiodinase (D2) was increased in BAT and aerobic muscle of Thra-0/0 mice. This and liver D1 were increased by a high-fat diet in both genotypes, as also were serum T\(_3\) and T\(_3\)/T\(_4\) ratio, but more in Thra-0/0 than WT mice (\(P < 0.001\)). Remarkably, when studied at thermoneutrality, genotype differences in weight and adiposity gain, respiratory quotient, D2, and LPL disappeared. Thus, disruption of BAT thermogenesis in Thra-0/0 mice activates an alternate facultative thermogenesis that is more energy demanding and associated with reduced fuel efficiency, leanness, increased capacity to oxidize fat, and relative resistance to diet-induced obesity, in all of which muscle LPL and deiodinases play a key role. (Endocrinology 149: 6471–6486, 2008)

Our laboratory has long been interested in understanding how thyroid hormone regulates thermogenesis and their physiological impact (reviewed in Ref. 1). The transgenic deletion of the thyroid hormone receptor genes (Thr) is not associated with readily evident disruption of temperature homeostasis (2, 3), which is probably why there has not been much interest in exploring thyroid hormone thermogenesis in such models. Although Thrb-deficient mice have no apparent problem regulating body temperature, the only phenotype consistently observed, common to all Thra-deficient models, is a 0.5–1°C lower core body temperature (2, 3). For this reason, we undertook the study of a transgenic Thra-deficient mouse that lacks all known products of the Thr, called here Thra-0/0 (4). [Thr, followed by a or b to indicate a or B, is the official abbreviation for the thyroid hormone receptor genes (GenBank) and will be used throughout. Most commonly used abbreviation has been TR, and this mouse was called in previous publications Thr-0/0. When citing the literature, we use the name of the receptors as published]. We have made important observations that underscore the importance of the temperature homeostasis mechanisms on energy economy, the importance of brown adipose tissue (BAT) as an energy-efficient thermogenic site, the importance of partition of fuel between tissues in energy expenditure, and the potential role of deiodinases in supporting other forms of thermogenesis and fat oxidation. Such observations are the focus of this report. Our first report on Thra-0/0 mice indicated that as any other Thr-1 knockout mice, and regardless of the genetic background, they have lower body temperature than appropriate wild-type (WT) genotype controls (4, 5). We further found that Thra-0/0 mice are cold intolerant (4–6°C) due to the inability of BAT to produce heat in response to norepinephrine. Interestingly, at room temperature (20–22°C), i.e. 8–10°C below thermoneutrality (30°C), when BAT contributes about 40% to total heat production, Thra-0/0 mice expend more energy (oxygen consumption and food intake) than WT controls, but this difference disappeared promptly after placing them at thermoneutrality, i.e. 30°C (5), suggesting that an alternate form of facultative or adaptive thermogenesis is recruited or activated to replace BAT thermogenesis, and furthermore, that this alternate facultative thermogenesis is more energy demanding yet less

Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.
effective, because it confers protection in cool but not in cold environments (4–6 C).

BAT is present only in mammals, including the human newborn and infant, and it is the major site of nonshivering facultative or adaptive thermogenesis (6, 7). In cold adaptation, BAT oxygen consumption may increase 20- to 30-fold, but the released energy is dissipated as heat instead of being captured in ATP (8), owing to a unique protein, BAT uncoupling protein 1 (UCP1), that allows the controlled uncoupling of phosphorylation of ADP from respiration. BAT’s critical role in cold adaptation is supported by a variety of experimental animal models made cold intolerant by disrupting BAT thermogenesis (reviewed in Ref. 7). Such capacity to dissipate energy as heat led to the idea that BAT could also be an antiobesity tissue, an idea that was pursued with great enthusiasm and that gained acceptance based on a variety of experimental evidence (9, 10). It was therefore a great surprise that transgenic mice lacking Ucp1 were not obese, although they were, as expected, cold intolerant (11). Moreover, Ucp1−/− mice gained less weight than the WT genotype when fed a high-fat diet and showed evidence of increased fat oxidation (11), whereas such a difference in weight gain was attenuated when ambient temperature was increased from 21 C to 27 C, suggesting that the increased capacity to oxidize fat was linked to cold adaptation and that BAT was not the only site of nonshivering thermogenesis (12), further supported subsequently by observations of gradual adaptation to cold in Ucp1+/− mice (13).

In this regard, Thra-0/0 mice represent another model of impaired BAT thermogenesis consisting of the inability to produce heat in response to adrenergic stimulation (5), but they differ from Ucp1−/− mice in that they spend more energy in environments below thermoneutrality (5). Thra-0/0 mice lack all known products of Thra and therefore may resort to different mechanisms from Ucp1−/− mice to compensate for the lack of BAT thermogenesis. Therefore, studying the phenotype of Thra-0/0 mice may reveal other alternative mechanisms and how they relate to the lack of Thra products. Besides, as recently discussed (1), the energy cost of homoeothermy is normally high, and Thra-0/0 mice could show how different thermogenic strategies could affect energy balance.

The work presented here describes our initial results in pursuing these issues. We specifically aimed to investigate the impact of the phenotype on energy demands and economy and how these are affected by experimental manipulations of ambient temperature and energy supply and to obtain insight into the mechanisms involved. The results support the concept that the energy cost of temperature homeostasis importantly taxes energy economy; that BAT has indeed evolved into an effective, energy-efficient thermogenic mechanism, as advanced by Liu et al. (12); and that there are other mechanisms that could replace BAT function. In Thra-0/0 mice, these seem characterized by a higher energy cost; a more important role for skeletal muscle as thermogenic tissue; increased fat oxidation, probably as the result of a different partition between muscle and white adipose tissue; and probably an important role for the iodothyronine deiodinases.

Materials and Methods

Animals

All experimental procedures, as well as housing, handling, and breeding protocols, were approved by both the Baystate Institutional Animal Care and Utilization Committee (protocol BMC 05-003) and that of L’Ecole Normale Superieure de Lyon, France, where some of the experiments were done. Thra-0/0 mice, lacking all known products of the Thra gene, were created in the laboratory of Dr. Samarut, as described elsewhere (4, 14). These mice were at the University of Chicago for several generations, back-crossed more than 10 times into the C57BL/6 background before Thra-0/0 male and female mice were transferred to our lab (15). We generated our colony by crossing Thra-0/0 with WT C57BL/6 breeders, and generated future generations of WT controls (i.e. Thra+/−) and Thra-0/0 mice from the inbreeding of the first generation of heterozygotes. Experiments performed in Lyon, indicated where appropriate, were done in the original Thra-0/0 mice bred into the 129S2 strain background. In both places, experiments were performed in 3- to 5-month-old males, ranging from 22–31 g at the start. For individual experiments, mice from each genotype were usually matched within 1 month of age and 5 g body weight, to minimize the confounding effect of age and size. Unless indicated otherwise, all experiments were performed with at least four mice per genotype and repeated at least once. Mice were kept at 21 ± 1 C, with a 12-h light, 12-h dark cycle starting at 0600 h and housed in standard plastic cages, usually five mice of the same sex per cage, over a commercial bedding of ground corncob (Haran Teklad, Indianapolis, IN). In experiments involving cold challenges or metabolic measurements (e.g. metabolic rate, oxygen consumption), mice were housed in individual cages. Specific ambient temperature manipulations are described with the individual experiments. Mice were normally fed a Rodent Teklad Global 2018 or a Teklad 7001 (from Harlan Teklad) diet, from now on called standard chow or simply chow. Both diets are very similar, containing approximately 3.3 kcal/g, with about 12, 60, and 19% of the calories from fat, carbohydrate, and proteins, respectively. We used two high-fat diets. The one used locally contained 5.24 kcal/g, 60% of the calories as fat, and 20% as protein and 20% as carbohydrate (Research Diets, Inc., New Brunswick, NJ) and is referred to as HiFat (diet), whereas the high-fat diet used in Lyon, dubbed Western diet (TD 88137 from Harlan Teklad), contains 4.5 kcal/g with 15.2% of calories as protein, 42.7% as carbohydrates, and 42% as fat.

The vast majority of the experiments were performed in C57BL mice, in Massachusetts, except when indicated in the text and figure legends.

In vivo measurements: energy expenditure

Energy expenditure was measured basically as described (5, 16), by indirect calorimetry in an open-circuit system (Qubits Systems, Kingston, Ontario, Canada). Mice were housed in individual, airtight plastic boxes through which air was pumped at a constant rate and where oxygen consumption (VO2) and carbon dioxide production (VCO2) were calculated from the corresponding concentrations in the air going in and out of the cage multiplied by the flow rate. Electrodes were calibrated before each experiment against commercial gas mixtures of accurately known composition. All experimental procedures, as well as housing, handling, and breeding protocols, were approved by both the Baystate Institutional Animal Care and Utilization Committee (protocol BMC 05-003) and that of L’Ecole Normale Superieure de Lyon, France, where some of the experiments were done. Thra-0/0 mice, lacking all known products of the Thra gene, were created in the laboratory of Dr. Samarut, as described elsewhere (4, 14). These mice were at the University of Chicago for several generations, back-crossed more than 10 times into the C57BL/6 background before Thra-0/0 male and female mice were transferred to our lab (15). We generated our colony by crossing Thra-0/0 with WT C57BL/6 breeders, and generated future generations of WT controls (i.e. Thra+/−) and Thra-0/0 mice from the inbreeding of the first generation of heterozygotes. Experiments performed in Lyon, indicated where appropriate, were done in the original Thra-0/0 mice bred into the 129S2 strain background. In both places, experiments were performed in 3- to 5-month-old males, ranging from 22–31 g at the start. For individual experiments, mice from each genotype were usually matched within 1 month of age and 5 g body weight, to minimize the confounding effect of age and size. Unless indicated otherwise, all experiments were performed with at least four mice per genotype and repeated at least once. Mice were kept at 21 ± 1 C, with a 12-h light, 12-h dark cycle starting at 0600 h and housed in standard plastic cages, usually five mice of the same sex per cage, over a commercial bedding of ground corncob (Harlan Teklad, Indianapolis, IN). In experiments involving cold challenges or metabolic measurements (e.g. metabolic rate, oxygen consumption), mice were housed in individual cages. Specific ambient temperature manipulations are described with the individual experiments. Mice were normally fed a Rodent Teklad Global 2018 or a Teklad 7001 (from Harlan Teklad) diet, from now on called standard chow or simply chow. Both diets are very similar, containing approximately 3.3 kcal/g, with about 12, 60, and 19% of the calories from fat, carbohydrate, and proteins, respectively. We used two high-fat diets. The one used locally contained 5.24 kcal/g, 60% of the calories as fat, and 20% as protein and 20% as carbohydrate (Research Diets, Inc., New Brunswick, NJ) and is referred to as HiFat (diet), whereas the high-fat diet used in Lyon, dubbed Western diet (TD 88137 from Harlan Teklad), contains 4.5 kcal/g with 15.2% of calories as protein, 42.7% as carbohydrates, and 42% as fat.

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mately 30°C for the mouse (17), is defined as the ambient temperature at which body temperature is maintained solely by obligatory thermogenesis, without the participation of heat-saving or dissipating mechanisms and of facultative thermogenesis. Thus, minimal VO2 values at thermoneutrality are the closest approximation to obligatory thermogenesis or basal metabolic rate, the energy spent to sustain vital functions. The average VO2 at 30°C, however, is higher than basal metabolic rate because it includes the energy expenditure associated with nonexercise physical activity such as displacements in the cage, grooming, etc. (17).

Food intake

Food intake, along with feces and urine collection, was measured in individual mouse metabolic cages (Mini Mitter; Respironics Co., OR) placed at the desired ambient temperature. Food consumed was recorded daily for at least 3 d, averaged, and expressed on a per-day and 100 g–0.75 body-weight basis, but not used to compare mice of grossly different body composition.

Core body temperature and physical activity

Core body temperature and physical activity were constantly monitored in free-moving mice for 24 h or longer, using telemetry equipment and software from Data Science International (DSI, St. Paul, MN). DSI TA-F20 transmitters were placed in the mice abdominal cavities through an approximately 1-cm incision, as recommended by the supplier. The procedure took a few minutes during which mice were anesthetized with 3–4% isoflurane in oxygen given at 500 ml/min with a loose snout mask. Abdominal wall was sutured in two planes, muscle with 4–0 absorbable suture and the skin with one or two metal clips. Mice were allowed to recover for at least 5 wk before experiments were performed.

Body composition

This was measured by nuclear magnetic resonance (NMR) in the experiments performed in Lyon, using the Minispec Fat & Lean Analysis device from Bruker Optics (Bruker, France). Where indicated, we also weighed the main white adipose tissue (WAT) depots.

In vitro measurements

In vitro measurements included RIA of serum T4 and T3, quantitative RT-PCR (qRT-PCR) of several mRNAs and type 1 5′-deiodinase (D1) and D2 activities.

Blood and tissue sampling

Blood was rapidly obtained from the inferior vena cava (usually 0.5–1 ml), under the isoflurane anesthesia described above, after which mice were killed by cervical dislocation to proceed to tissue harvesting. Blood was allowed to coagulate at room temperature, and cleanly collected serum was stored at −20°C until analyzed. Tissues were snap frozen in liquid nitrogen and kept at −80°C until analyzed. BAT and WAT depots were rapidly weighed before freezing.

Biochemical assays

Total T4 and T3 levels were measured by RIA using commercial kits (Diagnostic Products Corp., Los Angeles, CA) with the modifications described elsewhere to optimize the assay for mouse serum (19).

RNA isolation and PCR analysis

Tissue RNA was extracted with acidified guanidinium-phenol-chloroform (20), quantified by absorbance at 260 nm, and stored in diethylpyrocarbonate-treated water at −80°C until further analysis. Integrity of RNA was routinely verified by agarose gel electrophoresis. The mRNA levels from several genes (mentioned where appropriate) were measured by real-time qRT-PCR done in a MX4000-v4.20 thermocycler (Stratagene, Cedar Creek, TX). Gene sequences were obtained from GenBank mouse genome (http://www.ncbi.nlm.nih.gov/sites/ entrez?db= gene). Primers were designed (PrimeQuest http://www.idtdna.com/

| Target gene | Tissues | Accession no. | Primer sequence (5′–3′) |
|-------------|---------|---------------|-------------------------|
| ACoAC       | WAT, liver, skeletal muscle | NM_133904.1 | Forward AAT GGC ATC GCT GCG GTC AAG TAT | Reverse TTT TGA TTA TTG GGT CCT CCT GGG |
| Cyclophilin D | All | BC019778 | Forward AGC AGA TAG ATC CAG ACT GCA ACC | Reverse CCT TCT GCC TTC TTA AGA TCA GCC |
| CK          | Skeletal muscle | NM_007710 | Forward AGC ATC AAG GGT TAC ACT CTG CCT | Reverse ACA CGG GCT TGT CAA ACA GGA ACT |
| Ckmr2       | Skeletal muscle | NM_198415 | Forward TCC GAA ACA AGA TGA CAC CCA GTG | Reverse GGG TCA TAG CCA TTG TCG CCT AGT |
| Dio1        | Liver | NM_007860 | Forward CCT CTC AGG ACA GGA GTG CAT | Reverse AAT GAT GAA GAA ATC GGG TGG GGA GG |
| Dio2        | Skeletal muscle, BAT | NM_001050 | Forward GCT GTA TGA CTC GCT CAT TCT GTA | Reverse AAA GTC AAG AAG GTG GCA TCG GGC |
| Mitochondrial glycerol phosphate dehydrogenase | Skeletal muscle, liver | NM_010274.2 | Forward GTG ACA TTC TGG GAG GCG | Reverse GTG ACA TTC TGG GAG GCG |
| PL          | Skeletal muscle, WAT | NM_008509.2 | Forward TCT GGA CGG AGG ATG GCA A | Reverse TCA TCA TGA GCA GGT TGG CCA TGG CC |
| ME 1, NADP(+) -dependent, cytosolic | Liver | NM_008615.2 | Forward GCA GCT CTT CGA ATT AAG AAG A | Reverse CCA ACC ATA TCT CCT TCT GAT CAG TCT CC |
| PGC1α       | BAT, skeletal muscle | NM_008904 | Forward ACA GCT TCC TGG GTG GAT ATG AGT | Reverse AGA CTG TCC AGT GGT TCT GTG AGG A |
| PPARγ       | WAT | NM_011146.2 | Forward AGA TCC TCC TGG TTA ACC AAG AAG A | Reverse TCA TCA TGA GCA GGT TGG CCA TGG CC |
| SERCA1      | Skeletal muscle | NM_007504 | Forward TCT GTC ATC AAG CAC AAG CAT | Reverse ACA GCT TCC TGG GTG GAT ATG AGT |
| UCP1        | BAT | NM_009463.2 | Forward ACA GAG TGT CAT | Reverse CGG ACA ACA AGA GCT AGT AAA |
| UCP2        | Skeletal muscle | NM_011671.3 | Forward TGC AGC GCC ACA TGA GTC TT | Reverse ATG CTC TGA GCC TTT GTT GTT GAA |
| UCP3        | BAT, Skeletal muscle | NM_009464.3 | Forward AGA ATG TGC TGG AGT CCT ACC TGT | Reverse GGA GCG TTC ATG TAT CGG GTC TTT |

All qRT-PCRs were performed in C57BL mice, in Massachusetts.

TABLE 1. Mouse genes quantified by qRT-PCR: target genes, tissues investigated, accession numbers and primers
TABLE 2. Daily weight gain and food intake in WT and Thra-0/0 mice fed chow or HiFat diet (60% of calories as fat) at 21°C for 5 wk

|                | Weight gain (g/d) | Food intake (kcal/d) | Fuel efficiency (mg/kcal) | Feces, wet weight (% of food intake) |
|----------------|-------------------|----------------------|---------------------------|-------------------------------------|
| WT-chow        | 0.177 ± 0.006     | 9.8 ± 1.1            | 16.5 ± 0.8                | 27.4 ± 1.2                          |
| Thra-0/0-chow  | 0.051 ± 0.003a,b  | 11.8 ± 1.3a          | 4.6 ± 0.6a,b             | 24.1 ± 0.7a,b                       |
| WT-HiFat       | 0.360 ± 0.020a,b  | 10.0 ± 1.6           | 39.8 ± 6.5a,b           | 12.7 ± 0.4a,b                       |
| Thra-0/0-HiFat | 0.230 ± 0.027a,b  | 14.2 ± 0.8           | 16.5 ± 2.3a,b           | 11.5 ± 0.3a,b                       |

Results are from a replicate experiment of that summarized in Fig. 7 (n = 5 mice per group). Experiments were performed in Massachusetts with the Thra-0/0 genotype in the C57BL background and HiFat, as defined in Materials and Methods. Daily weight gains were calculated from the slopes of the individual weight gain curves, which were not different from those in Fig. 7A, from 0–35 d. Food intake was measured between d 32 and 35 and is expressed in kilocalories per day, with no correction by weight. Fuel efficiency was calculated by dividing individual weight gain slopes in milligrams per day by daily individual daily caloric intakes.

**Significant differences between genotype in the same diet.**

^1 Significant differences between genotype in the same diet.

^2 Statistical difference made by the HiFat diet for either genotype.

^3 P < 0.001.

^4 P < 0.01.

^5 P < 0.05.

**Scitools/Applications/Primerquest** to obtain products of 110-220 bp, spanning when possible at least one intron to control for eventual DNA contamination (we never detected it). Table 1 shows all primers with their corresponding accession numbers. Cyclophilin was used as housekeeping gene to correct for variability in mRNA loading, and results are expressed as ratios of test mRNA/cyclophilin mRNA abundance calculated from SYBR-Green fluorescence (Stratagene). Calculations were based on Ct, the number of cycles needed to reach an arbitrary threshold fluorescence level. This was set in the lower half of the linear part of product accumulation and was kept the same in all assays.

**Deiodinase activities**

They were measured basically as described (21), in whole homogenates of BAT and liver, using 125I-labeled rT3 as substrate. Before the assay, commercially obtained 125I-labeled rT3 was purified in a 1-ml bed volume column of LH-20 Sephadex (Sigma Chemical Co., St. Louis, MO) as described (22). For D2, assay conditions were 2 nm rT3, 20 mm dithiothreitol, and no PTU. Protein concentration and reaction times were adjusted to keep [125I]rT3 consumption less than 20%, usually 120–150 µg BAT or 60–80 µg liver homogenate protein for, respectively, 90 and 15 min. Activity was measured by the release of 125I-. Reaction was stopped by adding 200 µl horse serum containing 1 mm PTU, followed by precipitation with ice-cold 10% trichloroacetic acid and 30 µl approximately 40% Dowex 50Wx2, 200–400 mesh suspension in 10% glacial acetic acid. This procedure leaves in solution only the 125I-, trapping all the [125I]rT3 and other lesser organic contaminants containing 125I in the serum and resin. Blank [125I] release with this procedure was usually 1% or less of the total counts.

**Other tissue measurements**

DNA and tissue protein content were measured by standard methods (23, 24).

**Statistical analysis**

Results are expressed as mean ± sem. Experiments were repeated at least once in experimental groups of three to six mice, unless indicated otherwise. Experiments involving several treatment or time groups were analyzed by ANOVA followed by post hoc tests for multiple comparisons. Two-way ANOVA was used to compare the effects of treatments on two experimental groups (e.g. time of each genotype on a given diet). Individual means were then compared by the Bonferroni’s test, whether to compare the differences in response to the experimental variable between genotypes (in a TWANOVA) or to compare the effect of two levels of variable, e.g. two different temperatures within a genotype. For simplicity, statistical significance is expressed in the figures and in Table 2 with the letters a and b to indicate, respectively, significant differences between or within genotypes. Both a and b are followed by numbers to indicate the probability level for the null hypothesis: 1 for P < 0.05, 2 for P < 0.01, and 3 for P < 0.001.

**Results**

**Effect of ambient temperature on energy expenditure**

Results depicted in Figs. 1–3 are to demonstrate the effect of cooling the environment on energy expenditure and balance in Thra-0/0 mice. Figure 1 shows the effect on VO2 of two ambient temperatures, room temperature (20°C) and a temperature close to thermoneutrality (28°C). Mice were reared at room temperature and then acclimated for 2 d at 28°C before the measurements. Figure 1A shows a representative 24-h mean VO2 recording from three mice of each genotype. As expected, VO2 is higher at
Ambient temperature and food requirements

A

Food intake (g/day \times 100g^{-0.75})

| Temperature | WT | Thra-0/0 |
|-------------|----|----------|
| 22°C        | 10 | 8        |
| 11°C        | 8  | 5        |
| 4°C         | 5  | 2        |

B

Weight loss (g)

| Condition  | 0h | 12h | 24h |
|------------|----|-----|-----|
| Absolute   | 0  | 1   | 2   |
| Percent    | 0  | 2   | 3   |

Fig. 2. Cold adaptation increases food demands more in Thra-0/0 than in WT mice. Experiments were performed in Massachusetts, with the WT and Thra-0/0 genotypes in the C57BL genetic background. A. Food consumption in mice exposed to 22, 11, and 4°C for 24 h and fed standard diet (chow, as defined in Materials and Methods). All mice were 3–4 months old, did not differ in weight, and had been reared at 21°C, eating chow at all times. Reducing ambient temperature is associated with increased food consumption, but the increment is greater in Thra-0/0 than in WT mice. B. Weight loss in response to a 16-h exposure to 10°C without food: left pair of bars and right y-axis show absolute weight loss (grams), whereas the right bars and right y-axis show relative loss (percent). Initial weights were not significantly different. Statistical significance is as in Fig. 1.

20°C than at 28°C in both genotypes, the difference representing energy cost of keeping body temperature in an environment below thermoneutrality. However, the cooling-induced increase in VO2 was higher in Thra-0/0 than WT mice, in contrast with nearly superimposed tracings at 28°C. In normal rodents, after a few hours below thermoneutrality, facultative thermogenesis is largely if not solely BAT thermogenesis, whereas almost instantly after moving rodents from a cool or cold environment to thermoneutrality, BAT sympathetic stimulation and thermogenesis cease promptly (7, 17, 25). The magnitude of the ambient temperature-induced changes is better appreciated in Fig. 1B, where mean 24-h VO2 for each condition is shown. At 28°C, mean 24-h VO2 was virtually the same, about 200 ml × h^{-1} \times 100 g^{-0.75}, in both genotypes, consistent with our previous report and with our previous finding that obligatory thermogenesis is not affected by the disruption of Thra (5). At 20°C, VO2 was nearly 50% higher than at 28°C in WT, whereas the corresponding increment in VO2 in Thra-0/0 mice was 20% bigger than in WT mice (P < 0.001), also consistent with the previous report (5) and indicating that the phenotype has not changed since. Because Thra-0/0 BAT produces virtually no heat in response to adrenergic stimulation (5), we concluded that the higher increment in VO2 of Thra-0/0 between room temperature, and thermoneutrality represents the energy cost of an alternate, more energy-consuming form of facultative thermogenesis.

Food consumption is another indicator of energy expenditure. Fig. 2A shows food consumption as a function of ambient temperature. Consistent with the cold-dependent increased VO2, food consumption also increases as the ambient temperature becomes colder. In normal rodents, after hours of cold exposure, when BAT has been recruited, additional food consumption is largely accounted for by BAT thermogenesis (7, 17). As the ambient temperature becomes colder, Thra-0/0 mice not only augment food intake, but the increase is steeper than in WT mice. Thus, while food intake increased by about 45% from 22°C to 4°C in WT mice, it more than doubled in Thra-0/0 mice (P < 0.001). Consistent with the higher energy demand, Thra-0/0 mice were more sensitive to food deprivation in cool or cold environments. In a representative experiment illustrated in Fig. 2B, Thra-0/0 mice deprived of food overnight in a 10°C environment lost significantly more weight than WT mice, whether expressed in absolute or relative terms. Under more severe conditions, 4°C without food (Fig. 3), WT mice survived with only a modest drop in core temperature (to 34.8 ± 0.2°C, see inset), whereas Thra-0/0 mice died of acute hypothermia between 10 and 20 h. (Death by hypothermia was not an endpoint. The experiment was carried out overnight, and it was promptly stopped when two mice were found dead, about 20 h into the experiment, the next morning. The other three Thra-0/0 mice were in state of torpor and could not be saved by warming.) Note that before dying, Thra-0/0 mice had surges of temperature (marked by asterisks in Fig. 3), which probably represent periods of intense shivering.

Dependency of food intake for cold resistance

Fig. 3. Food intake is essential for survival in Thra-0/0 mice. Experiments were performed in Massachusetts, with the WT and Thra-0/0 genotypes in the C57BL genetic background. Mice were 4–5 months old. Groups of five Thra-0/0 or WT reared at 21°C were acutely exposed to 4°C for 24 h without food. Each line represents the core temperature of one mouse. Although all WT mice survived, undergoing a mild drop in body temperature (inset), Thra-0/0 mice became rapidly hypothermic and died between 10 and 20 h cold exposure. As indicated in text, death was not an intended endpoint. Experiment was started around noontime. Next morning, nearly 18 h later, two Thra-0/0 were found dead, and three were in a state of torpor and could not be rescued by warming. Note also that WT core temperature increased after reaching a nadir around 15–16 h, reflecting the recruiting of BAT, which did not occur in Thra-0/0 mice.
Nonexercise physical activity

Physical activity is associated with thermogenesis and increased energy demands. This is clearly evident in mice, as illustrated in Fig. 4 where representative tracings show the close correspondence between bursts of activity and increases in core body temperature in both WT and a Thra-0/0 mouse. Therefore, increased physical activity might be a way for Thra-0/0 mice to compensate for the lack of BAT thermogenesis, accounting in turn for their increased energy expenditure. Figure 5 shows that this is not the case. In this experiment, the numbers on the ordinate represent the combination of distance and speed of displacements integrated into 1-min intervals (i.e., mechanical work) over the time of observation indicated in the abscissa (visit www.datasci.com for further information). The black line is the mean of five WT mice, whereas the gray is that of five Thra-0/0 mice. Evidently, the tracings are superimposed, with no difference in the intensity, timing, or circadian rhythm of the activity (the normal increase during the hours of darkness). The 3-d mean activity was around 5 counts/min, as shown in the upper part of the graph. Figure 5B shows the relative frequency distribution of activity, divided into intervals (bins) of five, i.e. 0–5 counts/min, 5–10, etc. The relative frequency for each bin has been plotted at the center of it. The curves generated by both genotypes are superimposed. Along with Fig. 4, these results show that activity occurs in spurts; that mice are quiet about 60% of the time, i.e. with activity between 0 and 5 counts/min; and that neither the time active nor the intensity of activity is different between the two genotypes. Lower ambient temperatures did not bring up differences between the two genotypes either. In rodents, a behavioral response to defend body temperature in very cold environments includes decreasing physical activity, minimizing displacement, and adopting a curled position. The adoption of a semispherical body shape and staying on one site minimizes heat loss by irradiation to the air and ground (17).

FIG. 4. Correlation between core body temperature and physical activity measured by telemetry. Experiments were performed in Massachusetts, with the WT and Thra-0/0 genotypes in the C57BL genetic background. The figure represents individual tracings of a representative WT and a representative Thra-0/0 mouse. See Materials and Methods and Results for details.

FIG. 5. Differences in physical activity do not explain differences in energy expenditure between WT and Thra-0/0 mice. Experiments were performed in Massachusetts, with the WT and Thra-0/0 genotypes in the C57BL genetic background. A, Mean activities of five mice of each genotype recorded continuously over nearly 3 d. Left axis shows the number of events per minute. The mean ± SEM activity (events per minute) is shown in the left upper corner. B, Frequency distribution of activity. The number of events per minute has been divided in intervals of five, i.e. zero to five, five to 10, etc. The relative frequency for each interval (bin) of activity is shown on the left axis, plotted in the center of each interval or bin.
Body composition of WT and Thra-0/0 mice

The findings so far strongly support the concept advanced previously (5) that facultative or adaptive thermogenesis is more energy costly in Thra-0/0 than in their controls. We then addressed the implications of this for body composition, fuel efficiency, energy balance, and tolerance to high-fat diets. Body composition was measured by NMR, as indicated in Materials and Methods, in the Lyon laboratory, in 22 WT and 24 Thra-0/0 mice at 21 C, fed chow and ranging from 3–4 month of age. Results are shown in Fig. 6. ANOVA indicates that the differences, although not dramatic, are highly significant (P < 0.0001). In WT mice, fat constituted 15.3 ± 0.9% of body weight, whereas in Thra-0/0, it was 12.4 ± 0.8% (P < 0.01 by Bonferroni’s test), which was mirrored by a 3% higher lean body mass in these mice (P < 0.01, by Bonferroni’s test) with no difference in free fluid content. Thus, when reared at room temperature, a few degrees below thermoneutrality, Thra-0/0 mice eat more and yet are leaner than WT controls.

Response to a high-fat (HiFat) diet

Such features suggest that Thra-0/0 mice are less fuel efficient and could be resistant to diet-induced obesity (DIO). To test such hypotheses, we challenged them with high-fat-containing diets both at room temperature and at thermoneutrality. Rodent high-fat diets usually have 40–60% of their caloric content as fat and are typically associated with weight and adiposity gain (see Refs. 26 and 27 and reference therein). Separate experiments were carried out in our laboratory and in Lyon, with two different diets and with the Thra-0/0 genotype in two different genetic backgrounds.

Results of a 4-month-long experiment, conducted in C57BL mice reared at 20–21 C, comparing the two genotypes eating chow (3.2 kcal/g) or the HiFat diet (5.2 kcal/g, 60% as fat) are summarized in Fig. 7. Thra-0/0 mice gained less weight than the corresponding WT controls, regardless of the diet, but the difference was obviously more evident with the HiFat diet (Fig. 7A). During the experiment, body weight increased linearly with time (r² > 0.90), the slope of the regression representing the average daily weight gain (grams per day, Fig. 7B). Please note the small SEM of the slopes. Weight gain was about 40% lower in the Thra-0/0 than in the WT control mice, but the increase was in both genotypes markedly greater with the HiFat diet, so the absolute mean difference in weight between the two genotypes at the end of the experiment was more than 15 g in the HiFat diet, whereas it was about 5 g in those on chow (Fig. 7A). Differences in size, shape, and body
fat accumulation are evident in Fig. 7C. Note the abdominal bulging of WT in both diets (1 and 3), causing a spheroid shape in WT mice on HiFat. The weight gain correlated significantly lower RQ than WT. Because in well-nourished mice after 20 h without food (0.70–0.001) on regular chow and 0.758 on HiFat (P = 0.004).

Results are summarized in Table 2. For both genotypes, whereas at 21 C, results were similar to those in the latter diet. In these experiments (not shown in Table 2), we also tested females, and their weight gains were similarly affected by genotype and diet (in g/d): WT chow, 0.098 ± 0.009; Thra-0/0 on chow, 0.050 ± 0.003; WT on HiFat, 0.217 ± 0.015; and Thra-0/0-HiFat, 0.134 ± 0.011 (P < 0.001 for genotypes in either diet).

Effect of ambient temperature on tolerance to diet

If the lower fuel efficiency and reduced sensitivity to high-fat-induced obesity of Thra-0/0 is caused by the higher energy requirements of an alternate form of facultative thermogenesis to BAT, one would predict that such differences would disappear or be markedly attenuated at thermoneutrality, when facultative thermogenesis is turned off (17). Consequently, we examined the weight and adiposity gain to a high-fat diet in groups of six to seven mice kept at 21 C or 30 C for nearly 5 wk. These experiments were performed in Lyon in the 129S2 strain or 30 C for nearly 5 wk. These experiments were performed in Lyon in the 129S2 strain. adiposity was measured in kilocalories per day, not corrected by body weight. We did these calculations in a second experiment identical to that summarized in Fig. 7. As in that experiment, weight increment was linear with time during the time of observation. Mean slopes (in milligrams per day) were then divided by daily food intake measured for two consecutive days, approximately 5 wk into the experiment. Results are summarized in Table 2. For each genotype and diet, weight gain slopes were similar to those in the experiment of Fig. 7. Likewise, caloric intake was significantly higher in Thra-0/0 than in WT mice either on chow or HiFat diet. Therefore, fuel efficiency, estimated by the ratio of daily weight gain (milligrams per day) to the daily caloric intake was nearly 4-fold greater in WT than in Thra-0/0 mice when on chow and about 2.5-fold greater when on HiFat diet. These differences between the two genotypes cannot be explained by reduced intestinal absorption because the wet weight of feces expressed as percentage of food intake was, if anything, lower in Thra-0/0 than in WT mice (P < 0.05) with both chow and HiFat diet. The heavier feces in mice eating chow compared with the HiFat diet in both genotypes is most likely caused by the higher nonabsorbable fiber content in the former than in the latter diet. In these experiments (not shown in Table 2), we also tested females, and their weight gains were similarly affected by genotype and diet (in g/d): WT chow, 0.098 ± 0.009; Thra-0/0 on chow, 0.050 ± 0.003; WT on HiFat, 0.217 ± 0.015; and Thra-0/0-HiFat, 0.134 ± 0.011 (P < 0.001 for genotypes in either diet).

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Weight gain represents the linear regression (expressed as percent gain per day because in Massachusetts with the experimental group, whereas RQ and fat burned, calculated from the RQ as explained in the original paper (5), none of the genes known to participate in BAT facultative thermogenesis was underexpressed in Thra-0/0 mice, and they all responded normally to cold. Although the failure of BAT to respond to adrenergic stimulation needs additional work (currently being pursued in our laboratory), we emphasize here the results on other tissues’ gene expression that could sustain the alternate facultative thermogenesis and the associated phenotype of Thra-0/0 mice.

Gene expression in several tissues

There are multiple gene products that could contribute to the phenotypic differences described between WT and Thra-deficient mice. As described in the original paper (5), none of the genes known to participate in BAT facultative thermogenesis was underexpressed in Thra-0/0 mice, and they all responded normally to cold. Although the failure of BAT to respond to adrenergic stimulation needs additional work (currently being pursued in our laboratory), we emphasize here the results on other tissues’ gene expression that could sustain the alternate facultative thermogenesis and the associated phenotype of Thra-0/0 mice.

**TABLE 3.** Weight gain, RQ, and fat burned in Thra-0/0 and WT mice fed chow or HiFat diet at 30°C (thermoneutrality) for 3 months

| Genotype and diet | A, WT Chow | B, Thra-0/0 Chow | C, WT HiFat | D, Thra-0/0 HiFat | A vs. B | C vs. D | A vs. C | B vs. D |
|-------------------|------------|-----------------|------------|------------------|--------|--------|--------|--------|
| Weight gain (%/d) | 0.331 ± 0.021 | 0.283 ± 0.015 | 0.881 ± 0.0046 | 0.896 ± 0.037 | NS     | <0.001 | <0.001 | <0.001 |
| RQ                | 0.83 ± 0.006 | 0.91 ± 0.006 | 0.770 ± 0.003 | 0.79 ± 0.002 | <0.001 | <0.001 | <0.001 | <0.001 |
| Fat burned (% EE) | 57.6 ± 1.9  | 26.8 ± 1.9     | 77.3 ± 0.9 | 69.8 ± 0.9 | <0.001 | <0.001 | <0.001 | <0.001 |

Results are from a single experiment during which mice were fed chow or HiFat (60% of calories as fat) for 90 d. Experiments were performed in Massachusetts with the Thra-0/0 genotype in the C57BL background and HiFat, as defined in *Materials and Methods*. Weight gain was here expressed as percent gain per day because Thra-0/0 mice on chow in this experiment were significantly smaller despite being of similar age. Weight gain represents the linear regression (±SEM) of 18 time points (weights measured approximately every 5 d) with five mice per experimental group, whereas RQ and fat burned, calculated from the RQ as explained in *Materials and Methods*, are the mean ± SEM of 36 measurements in three (WT) and four (Thra-0/0) mice over 24–25 h. Data were analyzed by ANOVA, and statistical significance between selected pairs was obtained with the Bonferroni’s test, as explained in *Materials and Methods*. NS, Not significant.
TABLE 4. mRNA levels of several genes in various tissues of Thra-0/0 mice fed chow or HiFat diet at 21 C

| Tissue          | mRNA | Standard diet | HiFat diet | ANOVA | P value |
|-----------------|------|---------------|------------|-------|---------|
|                 |      | A, WT         | B, Thra-0/0| C, WT | D, Thra-0/0 |       |
| BAT             | CP1  | 125 ± 8       | 187 ± 30   | 191 ± 41 | 172 ± 36 | 0.4563 NS NS NS NS |
| Liver           | ME   | 9080 ± 1155   | 3949 ± 253 | 5800 ± 567 | 3346 ± 396 | 0.001 <0.01 <0.05 NS NS |
| Liver           | ACoAC| 360 ± 80      | 226 ± 47   | 504 ± 74  | 234 ± 39  | 0.005 NS NS NS NS |
| Liver           | mGPD | 130 ± 23      | 110 ± 38   | 258 ± 26  | 389 ± 111 | 0.0908 NS NS NS NS |
| WAT             | PPARγ| 3554 ± 176    | 3656 ± 370 | NS     |           |       |
| Epididymal      | sc   | 1736 ± 336    | 2313 ± 442 | NS     |           |       |
| WAT             | LPL  | 4385 ± 592    | 5212 ± 494 | NS     |           |       |
| WAT             | ACoAC| 3131 ± 577    | 2901 ± 475 | NS     |           |       |
| Epididymal      | sc   | 586 ± 74      | 1219 ± 124 | <0.001  |           |       |
| Skeletal muscle | UCP3 | 1897 ± 815    | 2008 ± 476 | 1858 ± 333 | 1911 ± 248 | 0.2500 NS NS NS |
| Skeletal muscle | UCP2 | 22.5 ± 2.1    | 40.0 ± 9.4 | 34.8 ± 2.6 | 41.4 ± 3.4 | 0.0395 NS NS NS |
| Skeletal muscle | SERCA1| 92 ± 15       | 72 ± 19    | 78 ± 10   | 30 ± 11   | 0.0361 NS NS NS |
| Skeletal muscle | ACoAC| 1217 ± 38     | 1286 ± 72  | NS     |           |       |
| Skeletal muscle | LPL  |               |            |        |           |       |

Results were obtained by real-time qRT-PCR and are expressed in arbitrary units normalized by cyclophilin A, i.e. as the ratio of test mRNA/cyclophilin A mRNA, each expressed in arbitrary units calculated from the time required to reach a low level of product as measured by fluorescence. Entries are the mean ± SEM of these ratios. Experiments were performed in Massachusetts with the Thra-0/0 genotype in the C57BL background and HiFat, as defined in Materials and Methods. Results are from three separate experiments: experiment 1 (Exp. 1), simultaneous chow and HiFat diet experiment (n = 3–5 per group) for 120 d; Exp. 2, only chow (Exp. 2a, three mice per group; Exp. 2b, eight mice per group); Exp. 3, simultaneous chow and HiFat diet for 70 d (n = 5 per group). In all experiments, mice were reared at 21 C and kept at this temperature for the whole experiment. Every sample was run in duplicate. In experiments 1 and 3, data were analyzed by ANOVA followed by Bonferroni’s test and in experiments 2a and 2b by unpaired Student’s t test. NS, Not significant.

phate dehydrogenase, a sensitive indicator of thyroid hormone action (28) was not significantly affected by either diet or genotype, so these results cannot be attributed to liver hypothyroidism. Besides, Thrb is the predominant receptor in liver (29), and ME expression is Thrb dependent (19, 30).

A recent paper reported that mice heterozygous for a Thra allele carrying a disabling mutation, PV, Trα+/PV mice, exhibited a leaner phenotype and signs of impaired adipogenesis and lipogenesis than the corresponding WT (Thra+/+). Such findings led the authors to propose that Thra1 was necessary for both adipogenesis and lipogenesis (31) and prompted us to examine WAT gene expression. As shown in Table 4, the Thra-0/0 genotype was not associated with the changes described by these authors (31). Although they found reduced expression of the genes encoding peroxisome proliferator-activated receptor-γ (PPARγ), lipoprotein lipase (LPL) or ACoAC, essential for, respectively, adipogenesis, lipid uptake, and de novo synthesis of fatty acids, we found these genes normally expressed in sc and epididymal Thra-0/0 WAT; if anything, ACoAC mRNA was significantly increased, not reduced, epididymal WAT (P < 0.001), which was not examined by Ying et al. (31). So, at variance with the Trα+/PV mice, Thra-0/0 mice do not show a pattern of gene expression suggestive of impaired adipogenesis or lipogenesis.

In the initial description of the Thra-0/0 phenotype (5), we hypothesized that skeletal muscle could be the site of the alternate facultative thermogenesis; hence, we examined here the expression of a number of candidate genes in the skeletal muscle of our mice. In the first HiFat experiment, we chose to investigate the tibialis anterior, because this muscle has a good mix of type I (slow) and type II (fast) fibers (5, 16). We did not find a significant effect of diet or genotype on the LPL mRNA was consistently increased in all muscles of Thra-0/0 mice examined, in several separate experiments. Interestingly, the relative levels of the LPL mRNA were 15–20 times higher in the soleus than in tibialis anterior, and higher in this than in gastrocnemius, underscoring the oxidative demands and use of fat as fuel by Type I fiber muscle. Note that this is a minimum relative difference between soleus and the other two muscles, as the soleus content of
cyclophilin mRNA was significantly higher, reducing the LPL/cyclophilin mRNA ratio. Thus, the level of expression of LPL, an indicator lipid oxidative activity of muscle, is in every case significantly higher in Thra-0/0 than in WT.

**Thyroid hormone deiodinases in Thra-0/0 mice**

Serum thyroid hormone levels in Thra-0/0 and WT mice fed Chow or HiFat diet are presented in Figure 10. Thra-0/0 mice have been reported to have normal circulating levels of T₃ and TSH, whereas the levels of T₄ have been found slightly reduced (2, 3). This reduction has no clear explanation and is so small under standard rearing conditions that it needs large numbers of animals to reach statistical significance (5, 32). Consistent with these previous observations, there was a trend of T₄ to be lower in Chow–fed Thra-0/0 mice, but we also observed a trend for serum T₃ to be higher, although neither difference reached statistical significance. The HiFat diet was associated with a significant increase in serum T₃ in both genotypes, whereas those of T₄ were significantly decreased. In the HiFat groups, there was a trend in Thra-0/0 mice to have higher serum T₃ and a significantly lower T₄ level than in WT mice fed HiFat (P < 0.05 Two way ANOVA + Bonferroni’s test). These HiFat-induced differences in serum concentrations resulted in a significant increase in the T₃-to-T₄ ratio, and notably, such increase was significantly greater (3 vs. 2.2, P < 0.01) in the Thra-0/0 mice. Altogether, these changes in serum thyroid hormone concentrations, associated with increases in D1 and D2 expression and activity (see below), are compelling evidence in favor of increased T₄ to T₃ conversion in response to HiFat diet, and with such conversion being more active in Thra-0/0 than in WT mice.

**Tissue deiodinases in Thra-0/0 mice**

Such findings prompted the examination of the two 5′-deiodinases in WT and Thra-0/0 mice. Results are shown in Fig. 11 and Table 4 (skeletal muscle D2 mRNA). D2 mRNA and activity were elevated in BAT of Thra-0/0 mice in a similar proportion (Fig. 11), but neither the levels of mRNA or activity nor the difference between the genotypes was significantly affected by the HiFat diet. Liver D1 mRNA and activity were significantly stimulated by the diet in both genotypes, and even though there was a trend to the increment being greater in the Thra-0/0 mice, it did not reach statistical significance.

Examining tissue distribution of D2 after its cloning of D2, Northern blot was not sensitive enough to detect the mRNA in...
mouse skeletal muscle (33), whereas with the more sensitive RT-PCR, D2 mRNA was readily demonstrable in this mouse tissue, as shown in Table 4. Interestingly, D2 mRNA levels, as LPL mRNAs, were higher in soleus (15–20 times) than in tibialis anterior or gastrocnemius muscle. The soleus, an aerobic, slow-twitch, type I fiber muscle, is more responsive to thyroid hormone and more important in thermogenesis (34). It should be noted that higher abundance of D2 mRNA in BAT, a prototype of D2-containing tissue, than in soleus (compare Fig. 11A with Table 4) is exaggerated by the much higher cyclophilin mRNA in soleus than in BAT. Despite the mRNA abundance in soleus, we could not reliably detect activity with our current assay, which may be due to low activity or more likely to another as yet unidentified factor. Two-way ANOVA and ANOVA of D2 mRNA in skeletal muscles indicated significant effects of muscle type and genotype. Soleus mRNA was significantly more abundant in Thra-0/0 than WT fed either chow or HiFat diet, and the level was further and significantly increased by HiFat in Thra-0/0 mice, whereas the stimulation by HiFat was not large enough in WT mice to reach statistical significance.

Discussion

The major issues emerging from results presented here are, first, the value of BAT as a thermogenic tissue; second, the impact of various thermogenic mechanisms, in this case activated to compensate for the lack of BAT thermogenesis, on energy balance; and last, the nature of such thermogenic mechanisms and how they could be affected by the lack of Thra gene products, which requires additional research beyond the scope of this paper and are currently being addressed in our laboratory.

Homeothermic species have been exposed to opposing pressures during evolution, namely the need to be less fuel efficient for the sake of temperature homeostasis and limited food supply, which has led to the selection of mechanisms to generate heat in the most energy-efficient manner (1). BAT has evolved as an efficient and effective thermogenic tissue by virtue of its capacity to produce heat and probably also because of its anatomical location around blood vessels and organs necessary for heat distribution and production. So far, two models of BAT thermogenesis disruption have been examined from the viewpoint of temperature homeostasis, the *Ucp1*+/− and our Thra-0/0 mice. Both models tolerate cool but not cold environments such as those they have to survive when living in the wild in latitudes far from the equator. In both models, impaired BAT thermogenesis is associated with less fuel efficiency and reduced sensitivity to DIO, supporting the concept of BAT as the most energy-efficient tissue for facultative (adaptive) thermogenesis. However, both models differ in the way they compensate for the lack of BAT thermogenesis, which will be further addressed in the discussion of our findings. The most important difference, probably related to the lack of Thra gene products, is that the compensation of Thra-0/0 mice seems to be more energy demanding than that of *Ucp1*+/− mice, so that the Thra-0/0 mice have clearly a higher energy expenditure than WT controls as they are in progressively colder environments, and their survival in the cold requires permanent availability of food. This supports the concept that mechanisms used for temperature homeostasis strongly influence energy balance.

The failure of BAT to produce heat in response to adrenergic stimulation (5) is the most important, if not the only factor in the cold intolerance of Thra-0/0 mice. In contrast with the hypothyroid state, where a reduction in obligatory thermogenesis contributes to the cold intolerance, this cannot be invoked in Thra-0/0 mice because obligatory thermogenesis, operationally defined by VO₂, at thermoneutrality, is normal in them. The disruption of BAT thermogenesis makes Thra-0/0 activate compensatory mechanisms that demand more energy than BAT to respond to cold. By definition, this thermogenesis is facultative because the difference in energy expenditure between Thra-0/0 and WT mice is not evident at thermoneutrality; it is made evident by cooling the environment and expands in proportion to the ambient coldness. Such a difference is great enough to make Thra-0/0 mice leaner despite their increased food consumption and less sensitive DIO when living a few degrees below, but not at, thermoneutrality. The excess energy expenditure is not the result of increased activity but the result of metabolic changes. The RQ of Thra-0/0 mice indicates that the alternate facultative thermogenesis uses fat preferentially as fuel, and the data suggest that such capacity may be related to increased fat uptake by skeletal muscle, particularly by more oxidative type of muscle, as well as to augmented activation of thyroid hormone by both D1 and D2, as will be further discussed below.

Why and how the lack of products of the Thra gene makes BAT thermogenesis unresponsive to norepinephrine is under study. As mentioned in a previous report (5), the responses to adrenergic stimulation of genes known to be essential for BAT thermogenesis, namely UCP1, PGC1, and D2, are not affected by the lack of Thra. If UCP1 is present and increases normally to adrenergic stimulation, it must be the activation of UCP1 that somehow fails to occur, i.e. the protein would not open to the protons in the intermembrane space to dissipate the gradient by reentering the matrix and generating heat (35). There is sufficient evidence to indicate that it is fatty acids that activate UCP1, although the exact mechanism is still debated (36, 37). Unpublished observations from studies in progress show that such failure is not caused by a defective cAMP generation or lipolytic response to adrenergic stimulation. For the moment, we can only state that somehow Thra is necessary for a key factor to activate UCP1.

As mentioned, both *Ucp1*+/− and Thra-0/0 mice share in common a reduced sensitivity to DIO and increased capacity to burn fat, but the phenotype of these two transgenics differs qualitatively and quantitatively. Obviously, such differences could derive from more diffuse effects of the lack Thra because the gene is expressed in many tissues and generates several products (38). Besides, Thra is expressed in many tissues before Thrb and is believed to play a role in development (2, 3, 38). In this regard, Ying et al. (31) recently reported that an inactivating mutation found in human Thrb causing thyroid hormone resistance, called PV, when introduced in the mouse Thra1, was associated with a leaner
phenotype, which the authors postulate is caused by the lack of Thra1 in WAT, resulting in reduced adipogenesis and lipogenesis, as evidenced by the reduced expression of key genes involved in these two processes, such as PPARγ (adipogenesis) and ACoAC (lipogenesis or de novo fatty acid synthesis). Such observations prompted us to examine gene expression in WAT of Thra-0/0 mice. As mentioned in Results, we investigated gene expression in WAT depots of Thra-0/0 mice and found all the genes that are underexpressed in TRα+/PV are normally expressed in Thra-0/0 mice, including PPARγ (adipogenesis and lipogenic enzyme gene expression), LPL (lipid uptake), and ACoAC (de novo fatty acid synthesis). If anything, this latter was overexpressed Thra-0/0 epididymal WAT. So our results do not support the concept put forth by Ying et al. (31) that the thyroid hormone-α is essential for adipogenesis and lipogenesis. Note that the homozygous TRαPV+/− genotype in Ying et al. (31) is lethal, in contrast to the total absence of Thra products, which suggest a gain of function with the PV mutation. Indeed, one allele with the PV mutation does exert a dominant-negative effect that is not only limited to the thyroid hormone receptor α or β but also to other genes as well, such as PPARγ (31), precisely a gene key for both adipogenesis and the expression of lipogenesis genes. Moreover, a previous report on the introduction in the mouse Thra gene of another inactivating mutation in the human Thrb of a kindred with thyroid hormone resistance resulted in the opposite phenotype of Thra+/PV, namely increased adiposity and sensitivity to DIO (39), also suggesting a gain of function by the mutated transgene. Although interesting and intriguing, such observations do not help to understand the role of normal Thra in energy balance.

Yet another mutation introduced in Thra1, this one causing a 10-fold reduction in the affinity of the receptor for T3, is associated with increased sympathetic stimulation of BAT and a leaner and obesity-resistant phenotype (40). Giving enough T3 to these mice, or crossing them with Thrb-deficient mice that have elevated levels of thyroid hormones, but not hyperthyroidism, abrogated the phenotype, leading to the conclusion that the unliganded Thra1 in the brain can cause central sympathetic stimulation. Although ingeniously addressing drawbacks of the above models, the authors cannot exclude a dominant-negative effect of the unliganded Thra1 in the euthyroid state, when it could interfere with the function of other nuclear receptors, as cited above for PPARγ (31). Nonetheless, these observations (40) might explain the increased sympathetic output seen in hypothryoidism (41), but they would not explain the spendthrift phenotype of Thra-0/0 because these mice, first, do not have Thra1 and, second, their BAT thermogenesis is impaired.

Findings more likely to be relevant to the phenotype of the Thra-0/0 mouse are the 2-fold increase in LPL expression in muscle, the increased D2 activity in BAT and in muscle, and the increased T3-to-T4 conversion mediated by D2 and D1 and exaggerated by the HiFat diet. Such a hypothesis is further supported by the observation that muscle LPL and D2 expression was several times higher in aerobic, type I muscle, such as soleus, than in fast-twitch anaerobic muscle and that the genotype effect was enhanced by the HiFat. Aerobic muscle plays a role in thermogenesis, particularly in species where BAT does not exist or is less important, such as birds and, among mammals, pigs, and such thermogenesis is thyroid hormone dependent (34, 42–44).

The evidence supporting a role of the increase LPL expression in the phenotype of Thra-0/0 is abundant. The limiting role of LPL in tissue uptake and metabolism of fat has been well demonstrated (see Ref. 45 for review). It has been proposed that LPL may play a role in the partition of food between storage (WAT) and oxidation (muscle) (45) and that the overexpression of LPL in skeletal muscle is associated with a leaner phenotype (46). Jensen et al. (47) have recently shown that transgenic mice selectively overexpressing LPL in muscle are more cold tolerant and oxidize more fat than controls. Increased expression of LPL on tissues with high oxidative metabolism and consumers of fatty acids, such as heart and skeletal muscle, can stimulate the consumption of triglyceride-rich lipoproteins, namely very-low-density lipoprotein and chylomicrons, and reduce triglycerides (48). The influence of fat oxidation in these tissues is also evidence in the opposite situation. Cardiac-specific knockout of the LPL gene causes increased triglycerides levels due to reduced clearance (49). Homozygous LPL knockout mice die during lactation of hypertriglyceridemia, and this phenotype can be rescued by its selective expression in skeletal muscle (50).

Some studies have gone further to show that the level of LPL expression correlates with the capacity of the cells to oxidize fatty acids such as acyl coenzyme A oxidase and carnitine palmitoyltransferase activities (49). Most relevant to our results are observations in transgenic mice with various levels of muscle LPL overexpression from the creatine kinase promoter (51). Although the highest levels of overexpression caused myopathy (20-fold increase in LPL activity), more moderate overexpression was associated with reduced weight gain, reduced adiposity, and increased peroxisomes and mitochondria (51), indicating that the LPL-dependent increase in fatty acids uptake can drive the capacity of muscle to oxidize them. Note that even though the regulation of LPL activity is complex and includes transcriptional, posttranscriptional, and posttranslational regulation (45), in all these studies, the increased LPL mRNA correlated well with LPL enzyme mass and activity. Altogether, these observations provide support to our proposition that the observed increase in LPL expression in skeletal muscle of Thra-0/0 mice may be playing a pivotal role in diverting fat for muscle thermogenesis rather than for WAT storage, providing the fuel for the alternate form of thermogenesis described, and being central to the lean, hypermetabolic, spendthrift, obesity-resistant phenotype of these mice.

The mechanism of the increase in LPL expression remains to be investigated. All we know is that it is cold dependent, as the rest of the phenotype, because the LPL mRNA difference between Thra-0/0 and WT mice acclimated at 30 °C was reduced from being at least 2-fold greater at 21 °C to 30% in those fed chow and disappeared in those fed HiFat (data not shown). However, the nature of the signal has to await further research. The majority of the studies on the physiological regulation of LPL expression and activity have focused on WAT, whereas studies in skeletal muscle are scarce. This is particularly true with regard to thyroid hormone.
Hypothyroidism is associated with translational stimulation of WAT LPL activity. A thyroid hormone-dependent protein binds to a regulatory element in the 3'-untranslated region of the LPL mRNA and inhibits its translation (52), so hypothyroidism causes increased LPL translation. Studies looking at LPL gene expression regulation by thyroid hormone are few, limited to BAT and heart, most of the time looking at hypothyroidism, with results that are hard to interpret and modest, inconsistent effects of hypothyroidism on LPL mRNA levels. Golozoubova et al. (53) studying cold adaptation in Ucp1−/− proposed that the muscle in these mice is in a state of chronic shivering in response to lack of BAT thermogenesis. Because muscle activity increases LPL (45), we cannot exclude the possibility that increased muscle tone or chronic shivering may play a role in increasing muscle LPL in Thra-0/0 mice. Lastly, food intake increases LPL activity, but in WAT, not in muscle (45), so the increased food intake in Thra-0/0 mice cannot explain the increase in muscle LPL. As noted before, we did not see LPL increased in WAT (Table 4).

The differences between Thra-0/0 and WT mice in liver D1 as well as in BAT and muscle D2 are also observations of potential relevance. The increased hepatic D1 mRNA and activity in HiFat-fed mice may be responsible for the increased serum T3 and T3/T4 ratio, indicating increased T4-to-T3 conversion. This response was significantly more vigorous in Thra-0/0 mice and may contribute to their higher capacity to burn fat. BAT D2 mRNA and activity were increased in Thra-0/0 mice, which we did not observe in our earlier studies, and may represent an adaptive response selected over years of inbreeding and contribute to improve increased overall T4-to-T3 conversion, as we have shown previously in rats (54). In addition to BAT, we found readily detectable D2 mRNA in skeletal muscle, but we were not able to detect activity reliably. However, skeletal muscle constitutes 40–50% of body mass, and this increase in D2 activity may contribute both to local as well as circulating T3. That low D2 activity per cell in a large tissue could be physiologically relevant is illustrated by human skeletal muscle D2 being a major contributor to circulating T3, despite the low specific activity of the enzyme (55). To the best of our knowledge, increased T4-to-T3 conversion caused by a high-fat diet has not been reported before. Elevated circulating levels of thyroid hormones have been reported in rats fed such diets, but the elevation is either centrally mediated or due to changes on carrier proteins, because both T4 and T3 are increased (56, 57).

Regarding T3 produced for local consumption, it has been demonstrated in all tissues containing D2, notably brain, anterior pituitary, and BAT, where this enzyme contributes at least 40% to the thyroid hormone receptor occupancy, even in a nonstimulated state (reviewed in Ref. 58). So, the levels found in muscle, particularly in aerobic muscle, where expression levels are 15–20 times higher than in glycolytic muscles, are likely to be of physiological significance as a local source of T3, and more so in Thra-0/0 than WT mice. As mentioned above, evidence supports a role for aerobic, slow-twitch, type I muscle fibers in thermogenesis, and this muscle is more responsive to thyroid hormone than other muscle fiber types (34, 43, 44, 59). Lastly, the difference in soleus D2 between genotypes disappears when mice are acclimated at 30 °C, regardless of the diet (data not shown), adding yet more evidence for a role in the alternate facultative thermogenesis of these mice.

In summary, the central focus of this report has been to investigate the impact of the lack of Thra gene products on energy balance and the possible mechanisms involved. As advanced in our previous publication (5), the failure of BAT to respond to adrenergic stimulation plays a central role. An alternate form of facultative thermogenesis is activated, and this makes Thra-0/0 mice have a greater cold-dependent increase in thermogenesis (and energy expenditure), which makes these mice be leaner, less fuel efficient, and more dependent on continued food supply for cold adaptation; use fat preferentially as fuel; and ultimately be less sensitive to DIO than appropriate WT controls. The findings, supported by a large body of literature, suggest that higher levels of LPL, D2, and D1 and increased T4-to-T3 conversion are at the core the alternate facultative thermogenesis of Thra-0/0 mice. Increased LPL in muscle but not in WAT would divert fat to the former tissue where its oxidation would be stimulated by higher levels of circulating and local T3. Ucp1−/− mice studied in L. P. Kozak’s lab show also cold-dependent reduced sensitivity to DIO and increased fat oxidation (12), and they advanced the concept that in the absence of BAT, other tissues may become site of facultative thermogenesis. However, the Ucp1−/− mouse model is more moderate, suggesting a role for Thra products elsewhere that allow the UCP1-deficient mice to be less energy spendthrift than the Thra-0/0 mice. These are very important observations. They support the concept that BAT thermogenesis has evolved as a very effective, efficient way to produce heat for temperature homeostasis and that when disabled, older, less effective mechanisms are recalled. Older thermogenesis mechanisms, more energy demanding, make temperature regulation much more dependent on constant food intake. Finally, the comparison between Thra-0/0 and Ucp1−/− mice shows how much various temperature homeostatic mechanisms (strategies) could affect energy requirements. This is an area that has never been investigated as a factor in the risk to developing obesity.

Acknowledgments

We are grateful to Nadine Aguiler and Pierre Contard (Lyon) and Elizabeth Henchey (Springfield) for their dedicated and efficient mice care and breeding as well as to Damien Jeantet (Lyon) for genotyping. This latter part was done in Springfield by P.P.

Received May 14, 2008. Accepted August 13, 2008.

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These studies have been partially funded by a grant from the American Thyroid Association, support from Baystate Medical Education and Research Foundation, l’Agence Nationale pour la Recherche (Grant ANR-06-BLAN230232-01), and the European Network of Excellence CRESCENDO.

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References

1. Silva JE 2006 Thermogenic mechanisms and their hormonal regulation. Physiol Rev 86:435–464
2. Forrest D, Vennstrom B 2000 Functions of thyroid hormone receptors in mice. Thyroid 10:41–52
3. Flamant F, Samarut J 2003 Thyroid hormone receptors: lessons from knockout and knock-in mutant mice. Trends Endocrinol Metab 14:85–90
4. Gauthier K, Plateroti M, Harvey CR, Williams GR, Weiss RE, Refetoff S, Willott JF, Sundin V, Roux JP, Malaval L, Hara M, Samarut J, Chassande O 2001 Genetic analysis reveals different functions for the products of the thyroid hormone receptor α locus. Mol Cell Biol 21:4748–4760
5. Marril H, Sellam A, Dany Tziany P, Gillis MA, Calderone A, Weiss RE, Samarut J, Silva JE 2005 Temperature homeostasis in transgenic mice lacking thyroid hormone receptor α gene products. Endocrinology 146:2872–2884
6. Hims-Hagen J 1986 Brown adipose tissue and cold-acclimation. In: Traylor P, Nichols DG, eds. Brown adipose tissue. London: Edward Arnold; 214–240
7. Cannon B, Nedergraad J 2004 Brown adipose tissue function: physiology and pathological significance. Physiol Rev 84:277–359
8. Foster DO 1986 Quantitative role of brown adipose tissue in thermogenesis. In: Traylor P, Nichols DG, eds. Brown adipose tissue. London: Edward Arnold; 31–51
9. Hims-Hagen J 1989 Brown adipose tissue thermogenesis role in thermoregulation, energy regulation and obesity. Prog Lipid Res 28:67–115
10. Lowell BB, Susucic VD, Hamann A, Lawitts JA, Hims-Hagen J, Kozak LP, Flier JS 1997 Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. Nature 367:640–742
11. Ennerbäck S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, Kozak LP 1997 Lack of mitochondrial uncoupling protein are cold-sensitive but not obese. Nature 387:90–94
12. Liu Z, Rosemblit M, McClaine J, Kozak LP, Zavacki AM, Ying H, Christoffolete MA, Aerts G, So E, Harney JW, Cheng SY, Larsen PR, Bianco AC, Weiss RE, Korcarz C, Chassande O, Cua K, Sadow PM, Koo E, Samarut J, Ribeiro MO, Carvalho SD, Schultz JJ, Chiellini G, Scanlan TS, Bianco AC, Thompson HL, Dreyer L, Sattler W, Bressol CL, Zechner R, Radeloff JV 2007 Cardiac-specific knock-out of thyroid hormone receptor-specific nuclear and extra nuclear actions. Mol Cell Endocrinol 213:1–11
13. Liu YY, Schwartz JB, Brent GA 2003 A thyroid hormone receptor α gene mutation (p938H1) is associated with visceral adiposity and impaired catecholamine-stimulated lipolysis in mice. J Biol Chem 279:38903–38920
14. Sytenko K, Sattler W, Bressol CL, Zechner R, Slawson JD, Davis RC, Weiss RE, Korcarz C, Chassande O, Cua K, MacLennan DH, Sadow PM, Koo E, Radesdorff J, Thompson HL, Dreyer L, Sattler W, Bressol CL, Zechner R, Radeloff JV 2007 Cardiac-specific knock-out of thyroid hormone receptor-specific nuclear and extra nuclear actions. Mol Cell Endocrinol 213:1–11
15. Zavacki AM, Ying H, Furuya F, Kato Y, Cheng SY 2007 Impaired adipogenesis caused by a mutated thyroid hormone α receptor. Mol Cell Biol 27:2553–2563
16. Macchia PE, Takeuchi Y, Kawai T, Cui K, Gauthier K, Chassande O, Seo H, Hayashi Y, Samarut J, Murata Y, Weiss RE, Refetoff S 2001 Increased sensitivity to thyroid hormone in mice with complete deficiency of thyroid hormone receptor α. Proc Natl Acad Sci USA 98:1318–1323
17. Croteau W, Whitemore SL, Schneider MJ, St. Germain DL 1995 Cloning and expression of a CDNA for a mammalian type III iodothyronine deiodinase. J Biol Chem 270:16569–16575
18. Williams & Wilkins 817–823
19. de ML, Arruda AP, Carvalho DP 2005 Role of sarco/endoplasmic reticulum Ca2+/-ATPase in thermogenesis. Biosci Rep 25:181–190
20. Herpin P, Lossiec G, Schmidt I, Cohen-Adad F, Duchamp C, Lefaucheur L, Goglia F, Lanni A 2002 Effect of age and cold exposure on morphological and functional characteristics of skeletal muscle in neonatal pigs. Pflugers Arch 464:610–618
21. Duchamp C, Barre H 1993 Skeletal muscle as the major site of shivering thermogenesis in cold-acclimated ducks. Am J Physiol Regul Integr Comp Physiol 265:R1076–R1083
22. Mead JD, Irvine SA, Ramji DP 2002 Lipoprotein lipase: structure, function, and role in disease. J Mol Med 80:753–769
23. Jensen DR, Schlaepfer IR, Morin PC, Pennington DS, Marcell T, Ammon SM, Biber E, Hummert-Hartmann J, Eckel RH 1997 Expression of diet-induced obesity in transgenic mice overexpressing skeletal muscle lipoprotein lipase. Am J Physiol 273:R683–R689
24. Jensen DR, Knaub LA, Konhilas JP, Leinwand LA, Maclean PS, Eckel RH 2005 Increased thermoregulation in cold exposed transgenic mice overexpressing lipoprotein lipase in skeletal muscle: an avian phenotype? J Lipid Res 49:870–879
25. Levak-Frank S, Weinstock PH, Hayek T, Verdery R, Hofmann W, Ramakrishnan R, Sattler W, Bressol CL, Zechner R 1997 Induced mutant mice expressing lipoprotein lipase exclusively in muscle have subnormal triglyceride and reduced high density lipoprotein cholesterol levels in plasma. J Biol Chem 272:17182–17190
26. Augustus A, Aysu PY, Hulat R, Temmink H, Berendsen A, Vinkamwadithayan RK, Park SY, Kim JK, Zechner R, Goldberg J 2004 Cardiac-specific knock-out of lipoprotein lipase alters plasma lipoprotein triglyceride metabolism and cardiac gene expression. J Biol Chem 279:29503–29507
27. Weinstock PH, Biskup CL, Alho-Setala K, Radner H, Ramakrishnan R, Levak-Frank S, Eiser E, Zechner R 1997 Severe hypertriacylglyceridaemia, reduced high density lipoprotein, and neonatal death in lipoprotein lipase knockout mice. J Biol Chem 272:18329–18334
28. Kern PA, Ranganathan G, Yuktah A, Ong JM, Davis RC 1996 Translational...
regulation of lipoprotein lipase by thyroid hormone is via a cytoplasmic repressor that interacts with the 3' untranslated region. J Lipid Res 37: 2332–2340
53. Golozoubova V, Hohtola E, Matthias A, Jacobsson A, Cannon B, Nedergaard J 2001 Only UCP1 can mediate adaptive nonshivering thermogenesis in the cold. FASEB J 15:2048–2050
54. Silva JE, Larsen PR 1985 Potential of brown adipose tissue type II thyroxine 5'-deiodinase as a local and systemic source of triiodothyronine in rats. J Clin Invest 76:2296–2305
55. Maia AL, Kim BW, Huang SA, Harney JW, Larsen PR 2005 Type 2 iodothyronine deiodinase is the major source of plasma T3 in euthyroid humans. J Clin Invest 115:2524–2533

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