Voluntary Exercise Adapts the Hypothalamus-Pituitary-Thyroid Axis in Male Rats

Rosa María Uribe,* Lorraine Jaimes-Hoy,* Candy Ramírez-Martínez, Arlene García-Vázquez, Fidelia Romero, Miguel Cisneros, Antonieta Cote-Vélez, Jean-Louis Charli, and Patricia Joseph-Bravo

Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca Morelos, México

The hypothalamic-pituitary thyroid (HPT) axis modulates energy homeostasis. Its activity decreases in conditions of negative energy balance but the effects of chronic exercise on the axis are controversial and unknown at hypothalamic level. Wistar male rats were exposed for up to 14 days to voluntary wheel running (WR), or pair-feeding (PF; 18% food restriction), or to repeated restraint (RR), a mild stressor. WR and RR diminished food intake; body weight gain decreased in the 3 experimental groups, but WAT mass and serum leptin more intensely in the WR group. WR, but not RR, produced a delayed inhibition of central markers of HPT axis activity. At day 14, in WR rats paraventricular nucleus-pro-TRH mRNA and serum TSH levels decreased, anterior pituitary TRH-receptor 1 mRNA levels increased, but serum thyroid hormone levels were unaltered, which is consistent with decreased secretion of TRH and clearance of thyroid hormones. A similar pattern was observed if WR animals were euthanized during their activity phase. In contrast, in PF animals the profound drop of HPT axis activity included decreased serum T_3 levels and hepatic deiodinase 1 activity; these changes were correlated with an intense increase in serum corticosterone levels. WR effects on HPT axis were not associated with changes in the activity of the hypothalamic-pituitary adrenal axis, but correlated positively with serum leptin levels. These data demonstrate that voluntary WR adapts the status of the HPT axis, through pathways that are distinct from those observed during food restriction or repeated stress. **(*Endocrinology* 155: 2020–2030, 2014)**

Energy homeostasis requires the adequate response of the hypothalamic-pituitary-thyroid (HPT) axis to stimuli; thyroid hormones regulate nonshivering thermogenesis, basal metabolic rate, and total energy expenditure (1–5). TRH, released from hypophysiotropic neurons of the paraventricular hypothalamic nucleus (PVN) into portal vessels in the median eminence, reaches the pituitary where it controls synthesis and secretion of TSH, which activates production and release of the thyroid hormones (THs) T_4 and T_3 (1–3). TH exert feedback effects at hypothalamic and pituitary levels, down-regulating pro-TRH and TSH synthesis and pituitary TRH-receptor (TRH-R)1, while, up-regulating expression of TRH-degrading ectoenzyme (pyroglutamyl peptidase II [PPII]) in tanycytes (2, 6–8). Other control points of HPT axis activity include regulation of T_3 tissue concentration by action of deiodinases (4, 9). Hypophysiotropic TRH neurons are metabolic integrators that fix the set point of the HPT axis; their activity is regulated by hormones, such as leptin, and neuronal afferents from the arcuate nucleus that act as sensors of the energy status of the animal and directly or indirectly modulate TRH expression (1–3, 10, 11). Energy-demanding situations, such as acute cold exposure or enhanced physical activity, increase rapidly and

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* R.M.U. and L.J.-H. contributed equally to this research.

Abbreviations: BAT, brown adipose tissue; BW, body weight; BWg, body weight gain; D2, deiodinase 2; DMH, dorso-medial hypothalamus; Exα, euthanized 3h after dark onset; HPA, hypothalamic-pituitary-adrenal; HPT, hypothalamic-pituitary-thyroid; ISH, in situ hybridization; MBH, medial basal hypothalamus; PF, pair-feeding/pair-fed; PPII, pyroglutamyl peptidase II; PVN, paraventricular hypothalamic nucleus; RR, repeated restraint; TH, thyroid hormone; TRH-R, TRH receptor; UCP-1, uncoupling protein 1; WAT, white adipose tissue; WR, wheel running.

transiently PVN pro-TRH mRNA levels and, concomitantly, the release of TRH, TSH, and THs (12–19). In contrast, the activity of the HPT axis is inhibited by acute stress (16, 17, 20–22). The rapid adjustment of the activity of TRH hypophysiotropic neurons, and TSH release, in response to various behavioral tasks depends on a balance between the stimulatory effect of increased physical activity and the negative impact of stress-induced enhancement of corticosterone serum levels (16, 17, 22).

Several events involved in energy homeostasis that are regulated by THs participate in exercise performance such as glucose and lipid metabolism, modulation of tissue-specific fatty acid uptake and oxidation, mitochondrial biogenesis and activity, and cardiac and respiratory function (2–5, 23, 24). The ability to cope with exercise is affected by thyroid status, because either hypo- or hyperthyroidism diminishes the tolerance or capacity to perform and recover (25, 26). There are conflicting reports regarding the response of the HPT axis to exercise; after an acute bout, it is rapidly activated (16–19, 27) but, depending on type and intensity of chronic exercise, serum TSH or TH levels may be unchanged or diminished due to exercise-induced stress or negative energy balance (28, 29). Energy deficit, as produced by fasting or food restriction, decreases the serum concentration of leptin, increases that of glucocorticoids, and produces tertiary hypothyroidism with reduced pro-TRH expression in the PVN, TRH release, serum TSH and TH levels (1–3, 10, 11, 30, 31). Chronic psychological stressors decrease serum TSH or TH concentrations (20, 32–34), but results regarding PVN pro-TRH expression are scarce and controversial (33, 34).

This work aimed to study the status of the HPT axis during voluntary wheel running (WR), a less stressful model than forced wheel or treadmill running (35–37), and a useful model with which to understand some aspects of human adjustment to exercise. We evaluated the dynamics of changes in the activity of the HPT and hypothalamic-pituitary-adrenal (HPA) axis, caused by up to 2 weeks of voluntary exercise, to detect events before permanent plastic changes due to exercise are established (37, 38). HPT and HPA axes data were compared with the response to repeated restraint (RR), a mild stressor in which food intake is decreased willingly (39). Because exercise reduces food intake and body weight gain, parameters that regulate the HPT axis (1, 3, 10, 30, 31, 36, 37), additional experiments included a sedentary group pair-fed (PF) to the exercise group.

Materials and Methods

Animals

Wistar male rats (260–330 g; 10–14 weeks old) from the Institute´s outbred colony were kept (2/cage), lights on 7:00 AM to 7:00 PM, water and food (Harlan 2018SX) ad libitum. Protocols followed the NIH guide for care and use of laboratory animals and were approved by the Institute's Bioethics Committee. Efforts were made to minimize animal suffering and reduce the number of animals used. Rats were euthanized by a trained person with a sharp guillotine in a separate room.

Restraint

Rats were introduced into a restraint tube or placed in a clean cage (controls) for 30 minutes (at 9:30 AM) (16), during 1, 7, or 14 days. After each session, rats were returned with their cage mates to avoid additional stress (35, 37). Weight and food intake were registered every 2 days. To detect persistent changes in gene expression or hormonal concentrations, animals were euthanized 24 hours after the last restraint session (32).

Exercise

Rats were placed individually into a plastic cage containing a wheel with a digital counter (AccuScan Instruments Inc; 25 cm diameter) or just bedding (sedentary group), with food and water, for 1, 3, 7, or 14 days without previous habituation, from 7:00 PM to 7:00 AM, and then returned with their cage mates. Revolutions (rev) were registered daily and body weight every 2 days at 7:00 AM; food (accounting for spilled food) and water intake were measured daily at 7:00 AM and 7:00 PM and provided immediately afterwards; additional experiments included a PF group provided with the amount of food consumed by the exercised group during dark or light cycles. Rats were euthanized 3 hours after light onset.

In another set of experiments, to detect responses corresponding to recent bouts of activity, rats were habituated to an inverted light cycle for 3 weeks, and either isolated or introduced into a wheel-containing cage during their dark-active period. Running behavior was videotaped during 3 days (days 7–9) to analyze time and amount of exercise performed by each animal; rats exercised more during the first hours of the active period in bouts of 40–65 seconds, with intermittent periods of feeding; by 3 hours after lights off, all rats displayed an average running activity; rats were thus euthanized on the 14th day, 3–4 hours after beginning of the active (dark) period (Exα).

Tissue dissection

Trunk blood was collected and brains were excised and stored at −70°C. Medial basal hypothalamus (MBH) was dissected either from frozen brain or fresh; the latter was dissected under a 3× magnifying lens, containing the median eminence, the arcuate nucleus and ventral portion of ventromedial nucleus, and immediately frozen. From frozen brain, MBH and dorso-medial hypothalamus (DMH) were dissected from a 1.3-mm coronal section (bregma, −2.3 to −3.6 mm (40) using a 0.5-mm or a 1-mm internal diameter (I.D.) sample corer (8) (Fine Science Tools) respectively; PVN from a contiguous slice (bregma, −0.84 to −2.3 mm) (16, 17). Average weight of one MBH dissected in fresh tissue: 8 ± 1 mg; from frozen punch: 15 ± 1 mg. Adrenals, interscapular brown adipose tissue (BAT), epididymal white adipose tissue (WAT), interscapular sc WAT, or retroperitoneal WAT, were weighed fresh, and frozen.

Hormones and triglyceride quantification

RIAs were employed for TRH as described elsewhere (16, 17), for TSH using NIDDK reagents (Bethesda, MD), and corticosterone (ICN), total T_3 (kit TKT31), and total T_4 (TKT41) from Siemens Diagnostic Products Corp by ELISA: leptin (Crystal Chem Inc); total T_4, T_3, and free T_4 (Diagnóstica Internacional). Intra- and interassay variation coefficients were less than 10%. Triglycerides were quantified in sera with strips (Accutrend Plus, Roche Diagnostics GmbH).

Semiquantification of mRNA levels

Total RNA was extracted from frozen tissues (17, 41), RNA purity was verified, and relative mRNA levels were measured by RT-PCR (Eppendorf Mastercycler Gradient) as described in References 16 and 17 and in Supplemental Material and Methods.

In situ hybridization analysis (ISH)

Frozen coronal slices (20 μm) from anterior part of PVN to caudal end of VMH (−1.08 to −3.96 mm) were cut in a cryostat, hybridized with an oligonucleotide (317–367 b pro-TRH), and quantified as described elsewhere (14).

Histology of adipose tissue

BAT or WAT pieces fixed in 5% formaldehyde were embedded in paraffin, sliced (10 μm sections), and stained with hematoxylin-eosin. Adipocyte size was analyzed visually, and cell density was analyzed with Explora Nova Mercator software, version 4.10 (42).

Activity of deiodinases

Frozen tissues were homogenized 1:10 wt/vol in phosphate buffer and centrifuged at 10 000 × *g* for 10 minutes (S2). Microsomal/membrane preparation were obtained from BAT or liver after 60 minutes centrifugation of S2 at 100 000 × *g*. Deiodinase 2 (D2) activity was measured using 150×10^5 cpm $^{125}IT_4$ as substrate (PerkinElmer, NEX111H) and either 70–80 μg protein of MBH-S2 supernatant and 90 minutes incubation, as described elsewhere (43), or 10 μg protein of BAT microsomes and 60 minutes incubation time (44). D1 activity was determined in liver microsomes (10 μg protein) with [^{125}I]rT3 as a substrate (PerkinElmer, NEX109) as described previously (44). All reactions included blank samples without homogenate; labeled hormones were purified before use with Sephadex LH 20 (45) (Sigma-Aldrich); released ^{125}I was separated with Dowex 50WX2, l00–200 mesh (Bio-Rad Life Science). Assays were optimized by testing different protein concentrations and incubation times to assure linearity and verified if the expected changes in activity were detected in MBH from starved animals, or in BAT from cold-exposed rats. Proteins were determined with Bradford protein assay (Bio-Rad).

Statistical analyses

The mean of duplicate determinations, or of 5–6 ISH quantifications per rat brain region was taken as one value; results were calculated as percent of each experiment's control group mean and pooled from at least 2 independent experiments. Results were analyzed by one- or two-way ANOVA (data in Supplemental Table 1), followed when significant ($P < .05$) by Bonferroni or Least Square Means post hoc test (Super Anova, Abacus Concepts, Inc; 4.57); unpaired *t* test (τ) and linear regression analyses were performed with Stat view. Reults are expressed as mean ± SEM.

Results

Dynamics of changes produced by exercise or restraint

The amount of daily voluntary exercise performed varied among individuals but remained stable as time progressed (441 ± 28 meters/night; 5 independent experiments, n = 31). Body weight gain (BWg) and food consumption diminished during the 14 days of exercise or RR compared with controls; food efficiency was also strongly decreased by exercise or RR (Supplemental Figure 1, a-d) (36–38). Rats ate more during the dark than the light phase, proportional to water intake and amount of exercise (Supplemental Figure 1, e–g) (36, 37, 46, 47). Exercise induced loss of scWAT and epididymal WAT mass and adipocyte size at 7–14 days, and of BAT mass since day 3, proportional to revolutions (47); restraint decreased only BAT mass (Supplemental Figure 2, a–f). Leptin serum levels diminished after 14 days of exercise, more than after restraint (48, 49) (Supplemental Figure 2g).

Corticosterone serum values increased after the first day of restraint and the first night of isolation (37) but values normalized by day 3. Restraint increased adrenal weight and PVN CRH-R1 mRNA levels while decreasing glucocorticoid receptor mRNA levels in PVN (Supplemental Table 3) (50–52). Exercise produced no significant changes in the stress axis (Supplemental Table 3), corroborating the fast adaptation to voluntary running (35, 37, 38).

A single restraint session increased PVN pro-TRH mRNA levels 24 hours later (Figure 1A), probably as a compensatory consequence of diminished PVN pro-TRH expression, TSH release, and serum T_3 concentration, which is detected in rats euthanized 45 minutes after the restraint session (16). The activity of the HPT axis was slightly inhibited after 1 day of exercise, with a significant drop in serum T_3 concentration (Figure 1C), and serum T_3:T_4 ratio (sedentary: 0.173 ± 0.017; exercise: 0.113 ± 0.001, n = 10/group; τ: $P = .015$). Exercise for 1 week decreased PVN pro-TRH expression, which remained low thereafter; MBH TRH levels were unaltered, and TSH serum concentration decreased at day 14. T_3 or T_4 serum levels remained normal (Figure 1C). Diminished PVN pro-TRH mRNA and serum TSH levels were unlikely due to stress because all HPT parameters were normal after 7–14 days of RR (Figure 1, A and C).

Regression analyses revealed that PVN pro-TRH expression correlated positively with BWg in most groups

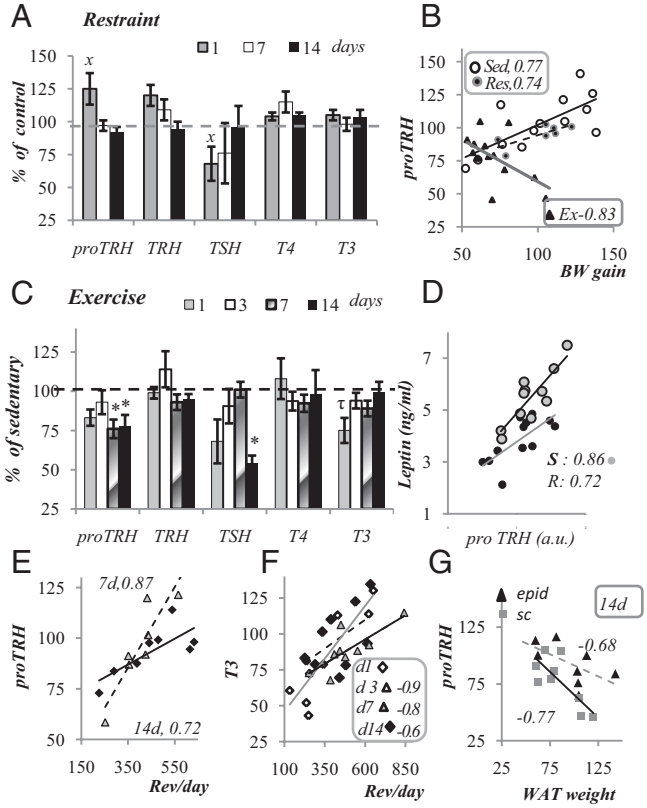

Figure 1. Effect of RR, or voluntary exercise on the activity of the HPT axis. Wistar male rats were introduced into a plastic restraint tube in prone position (Res), or in an empty cage (controls, C), for 30 minutes/d during 1–14 days. Male rats from another cohort were kept during the night in individual cages containing (exercise, Ex) or not a running wheel (sedentary, Sed), and returned with their cage mates during the light period. Each time point was repeated in 2 independent experiments (n = 10 rats per group); animals were euthanized at 10:00 AM. Data are percent of mean value in control or sedentary animals. A, Time course of changes in the levels of: PVN pro-TRH mRNA (ratio proTRH cDNA/cyclophilin cDNA), amount of immunoreactive TRH in the MBH (5.4 ± 0.1 ng in controls), and serum concentration of TSH, T_4, and T_3 of rats restrained during 1, 7, or 14 days and euthanized 24 hours after last session. C, As in panel A, but after 1, 3, 7, or 14 days of daily voluntary exercise. Serum concentration of controls: TSH, 5.7 ± 0.5 ng/mL (n = 48); T_4, 5.13 ± 0.17 μg/dL (n = 25); T_3, 84 ± 3 ng/dL (n = 18). Significant ANOVAs (Supplemental Table 1) followed by post hoc: *, significant differences against controls (C or Sed) of same day, ^{x}, $P < .05$–0.01; *, $P < .01$; **, $P < .001$); τ, significance by paired *t* test vs controls. B, D, and G, Correlation plots between variables stated in axes (expressed all as percent of sedentary except for D), from groups depicted in inserts according to symbols; values of Pearson coefficient in insert ($P < .005$). a.u., arbitrary units; Rev, revolutions.

(except negatively, at day 14 exercise) (Figure 1B) and with leptin serum levels (Figure 1D). Levels of PVN pro-TRH mRNA (Figure 1E), T_3 (Figure 1F), TSH or T_4 (0.53 or 0.54; $P < .05$) in serum correlated positively with revolutions whereas only those of pro-TRH or T_3 correlated negatively with WAT mass (Figure 1G and Supplemental Figure 2k). As reported, WAT mass varied negatively with PVN pro-CRH mRNA levels but varied positively with serum corticosterone levels (Supplemental Figure 2, h and i) (52, 53).

State of the HPT axis after 2 weeks exercise or PF

Because both exercise and restraint caused a similar slight negative energy balance, but loss of adipose tissue only in the former, we compared the status of the HPT axis in rats exercised for 14 days with that of sedentary PF rats, or fed ad libitum (sedentary). Exercised or PF animals had similarly reduced food intake and food efficiency (Supplemental Figure 1, c and d). The PF rats ate all the food provided; they finished it almost immediately after lights on and seemed anxious. BWg diminished similarly in exercised and PF rats; however, exercise reduced the weight of various WAT types whereas PF reduced only that of scWAT (Figure 2A).

To evaluate whether changes produced by exercise were affected by the time delay between the activity period and death, an independent set of experiments was performed with animals euthanized 3 hours after the beginning of the active period. Consistent with loss of WAT weight, triglycerides and leptin serum concentrations diminished after exercise (27, 54), more pronouncedly in animals euthanized in their resting period (after light onset) (Figure 2, B and C). In contrast, serum corticosterone levels were increased 3-fold in the PF group compared with the Ex and Sed groups; levels in Exα rats were slightly increased compared with their sedentary controls (Figure 2D). Variations in serum corticosterone values in PF rats correlated positively with WAT levels (Supplemental Figure 2j).

The food restriction imposed on the PF group was only 18 ± 1%, compared with sedentary rats, but sufficient to decrease PVN pro-TRH mRNA levels more than exercise; pro-TRH mRNA levels were inversely proportional to serum corticosterone levels, as seen in restraint (Supplemental Figure 2, l and m). Exercise-induced decreases in the levels of PVN pro-TRH mRNA or serum TSH in the Exα group were similar to those detected in animals euthanized after light onset. T_3 serum levels decreased only in the PF group. No changes were detected in free or total T_4 concentration, but the T_4:TSH and free T_4:TSH ratios were higher in PF than in exercised rats (Figure 2, E and F).

Additional markers of HPT activity after 2 weeks of exercise in rats euthanized during the light phase

Tanycytes control T_3 levels that reach PVN neurons through the activity of D2, as well as the amount of TRH that reaches the pituitary through the activity of PPII (8, 9). MBH D2 mRNA levels increased 50% in exercised rats (Figure 3A). The increase in MBH-D2 activity after exer-

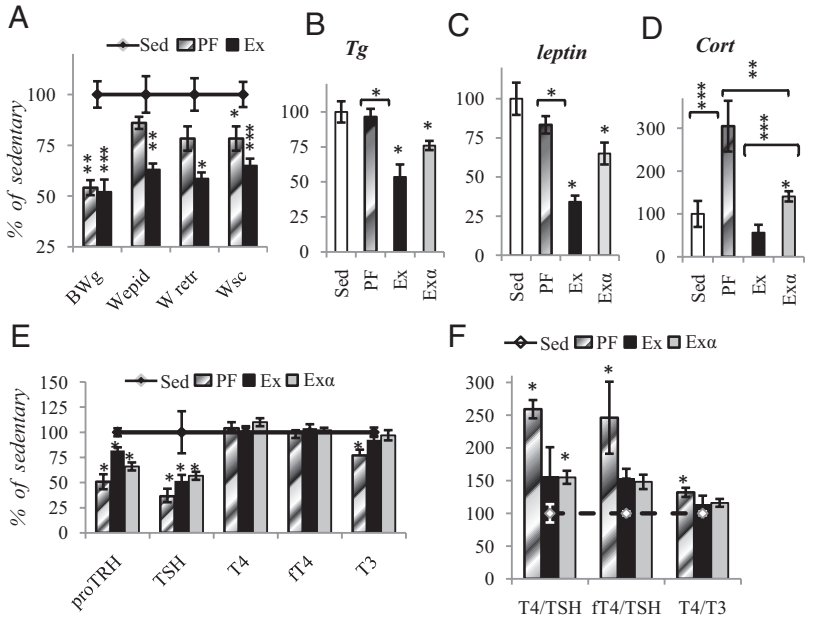

Figure 2. Effect of exercise, or PF on HPT axis and fat reserves at day 14. Male Wistar rats were kept during the night in individual cages containing (exercise, Ex) or not a running wheel (sedentary, Sed) and returned with their cage mates during light period. In one type of experiments, a group of rats was fed with the same amount of food as the exercised rats (PF), Sed (n = 12) and Ex (n = 12) were euthanized 3 hours after light onset (day). In another, the sedentary group (n = 8) and exercised rats (n = 7) were euthanized 3 hours after beginning of activity (lights off, night) (Exα). Results are expressed in percent of mean value of sedentary rats of each experiment. A, Histogram shows data of BWg, weight of WATs (W) (epididymal [epid], retroperitoneal [retr], and interscapular [sc]). B, Serum concentration of triglycerides (Tg); values in Sed euthanized during the day (D): 376 ± 64, night (N): 268 ± 19 mg/mL. C, Serum concentrations of leptin, Sed, D: 5.8 ± 0.4; N:4.2 ± 0.5 ng/mL (n = 8) D, Serum concentration of corticosterone (CORT), D: 117 ± 15; N: 216 ± 23 ng/mL. E, Status of the HPT axis in exercise (Ex or Exα) or PF rats; TSH: D: 3.28 ± 0.4; N: 3.5 ± 0.8 ng/mL; T_4, D: 6.5 ± 0.3; N: 7.13 μg/dL; fT_4, D: 2.15 ± 0.5; N: 2.1 ± 0.3 ng/dL; T_3, D: 10.6 ± 0.5; N: 9.8 ± 0.3 ng/mL. F, Ratio of individual values of hormones depicted in abscissa. Significant ANOVAs (Supplemental Table 2) followed by post hoc: *, significant differences against Sed of each experiment, $P < .005$: ^{x}, $P <$ .05-.01; *, $P < .01$; **, $P < .001$.

cise or PF was barely significant when expressed by total activity (Figure 3B) but not by specific activity because the differences in protein of surrounding tissue not expressing D2 increases variability. MBH PPII mRNA levels were not altered by exercise (Figure 3A). At the anterior pituitary level, among those genes involved in modulating TSH serum concentration as TRH-R1, D2, or TSH-β (2, 7, 9), only TRH-R1 mRNA levels increased after exercise (levels in PF rats were not measured) (Figure 3C).

Brown adipocytes are targets of THs and of sympathetic innervation. BAT weight decreased after exercise or restraint, but not after PF (Figure 3D). Decrements in BAT weight coincided with reduced size of lipid droplets and increased nuclear density by 63% (Supplemental Figure 2f), suggesting increased lipolysis. The expression of molecules involved in thermogenesis as D2 and uncoupling protein 1 (UCP-1), was differentially regulated; that of D2 increased after 14 days of exercise whereas that of UCP-1 decreased in the PF group (Figure 3D). BAT D2 specific activity was not significantly modified in exercised rats but decreased in the PF group (Figure 3E); in contrast, that of D1 in liver was inhibited in the PF group, coincident with low T_3 serum levels (Figure 3F).

Effect of exercise and PF on pro-TRH expression in hypothalamic nuclei

Measurement of pro-TRH mRNA levels by ISH in some hypothalamic nuclei demonstrated that the inhibition caused by exercise, detected by RT-PCR, occurred in the medial PVN; in addition, levels tended to increase in the DMH and were not altered in the lateral hypothalamus (Figure 4A). Because DMH is important for energy homeostasis (55, 56), we quantified pro-TRH and pro-CRH mRNA levels in response to PF or exercise. Pro-CRH mRNA levels increased by exercise (Figure 4B) as reported elsewhere (57). As observed by ISH, pro-TRH mRNA levels not significantly increased by exercise; however, they were decreased in the PF group, whereas an increase was detected in the Exα rats (Figure 4C).

Discussion

Adjustments of the HPA and HPT axes to different stimuli lie at the basis of energy homeostasis. Exercise requires an adequate energy distribution of fuels to oxidative tissues, eg, muscle and heart, which involves concerted changes in metabolism (5, 58, 59). We evaluated the status of the HPT axis in rats submitted to voluntary exercise for 2 weeks, in rats submitted to a mild chronic stressor as restraint, and in rats with restricted food intake, as caused by exercise (PF), in order to distinguish the contribution of stress and decreased energy intake to exercise data. Food intake, body weight gain, food efficiency, and BAT weight diminished in all treatments (35–37, 46–53), but levels of PVN pro-TRH mRNA and serum TSH decreased only in the exercised and PF groups whereas those of serum T_3 decreased only in PF rats.

Several additional differences were noted between paradigms. Exclusive to exercise was a decrease in WAT

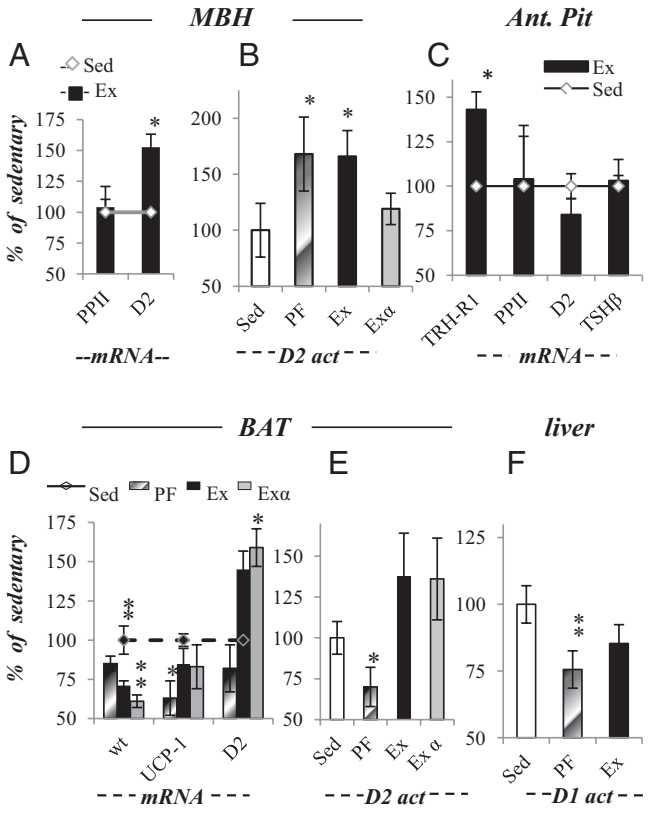

Figure 3. Effect of exercise, or PF, on the expression and activity of other HPT status-related genes. Exercised, PF, and sedentary rats, as described in Figure 2. Results are expressed in percent of mean value of sedentary rats. A and C, Relative mRNA levels of PPII and D2 measured in MBH (A) and in anterior pituitary (C), of TRH-R1 and TSH-β by RT-PCR calculated as the ratio of cDNA/cyclophilin cDNA and in BAT: of UCP-1 and D2 calculated as cDNA/HPRT cDNAs. Deiodinase activities: B) D2 total activity of MBH 10 000 $\times$ *g* supernatant (specific activity of SED: 0.31 $\pm$ 0.08 fmol/min/mg prot), E) D2 activity in BAT microsomal fraction, SED: 4.3 $\pm$ 0.8 fmol/min/mg prot; and F) D1 activity in liver microsomal fraction, 62 $\pm$ 5 pmol/mg prot/min. *, P = .008. Ant. Pit., anterior pituitary; wt, weight.

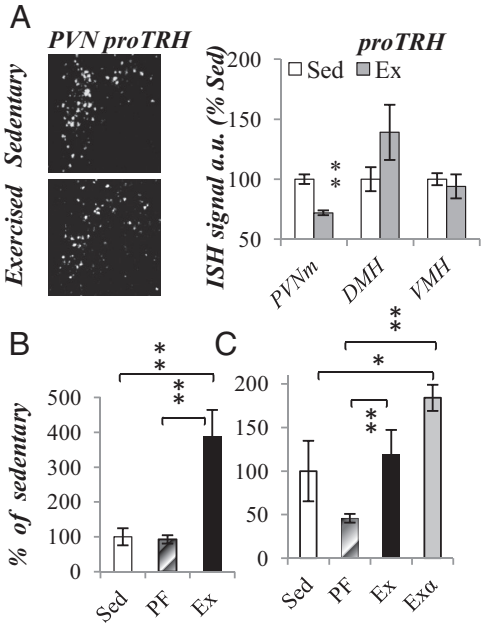

Figure 4. Effect of exercise or PF on gene expression in hypothalamus. Exercised (Ex), PF, and sedentary (SED) rats, as described in Figure 2. Results are expressed in percent of mean value of sedentary rats. A, Dark field illumination images of ISH of proTRH mRNA in the medial PVN (mPVN) region of sedentary and exercised rats; right panel: ProTRH ISH signal intensity in the PVN, DMH, and ventromedial hypothalamus (VMH) of sedentary and exercised rats at day 14, euthanized 3 h after light onset. C, Relative levels of proTRH mRNA (proTRH cDNA/cyclophilin cDNA) in the DMH of Sed, PF, Ex, or 3 h after beginning of activity. B, Relative proCRH mRNA levels (proCRH cDNA/cyclophilin cDNA) in the DMH of sedentary (Sed), pair-fed (PF), or Ex rats.

weight (35, 46–49) that led to a proportional drop in circulating leptin concentration (47, 48). Although we only measured scWAT mass after restraint, it is well documented that body weight (BW) loss is due to lean mass, at least during the first days (49, 60); the lowering of leptin levels, if due to lowering of epididymal WAT or, to diminished food intake, awaits explanation but it did not seem sufficient to affect levels of PVN pro-TRH mRNA or serum TSH. After 2 weeks of restraint, serum corticosterone levels were normal but changes in the expression of glucocorticoid receptor and CRH-R1 mRNAs in the PVN or in adrenal weight supported a state of chronic stress (49, 50–52) that did not affect the central arm of the HPT axis. In contrast, PF rats had a 3-fold increase in circulating corticosterone levels that could reflect a situation of high stress induced by hunger or expectation of food delivery (61), because rats had finished their food 2 hours before death. Similar published results with rats submitted for 2 weeks to chronic variable stress support our findings (62); stressed animals had normal serum corticosterone and decreased leptin levels compared with the weight-matched group that had lower decrement in leptin but high corticosterone levels. Because glucocorticoids induce WAT deposition, further evidenced by the positive correlations between WAT mass and serum corticosterone levels in PF and restraint rats, the reduced BWg is largely produced by loss of lean mass (49, 52, 53, 62). Only in the PF rats did T_3 serum levels decreased, as reported for several protocols of caloric restriction (1, 30, 31, 63). Even after 1 year in men with a 16%–20% calorie restriction, equivalent to the decreased voluntary food intake of exercised men, serum levels of T_3 are decreased, but not those of T_4 or free T_4, compared with healthy sedentary controls; by then, leptin and TSH serum levels and WAT mass loss are similar in calorie-restricted or exercised subjects (64).

The decreased activity of the HPT axis caused by 2 weeks of 18% food restriction or by exercise does not seem to follow common causes. Leptin regulates PVN pro-TRH transcription directly in TRHergic hypophysiotropic neurons and/or indirectly through the arcuate nucleus, inhib-

iting the activity of neuropeptide Y and Agouti related peptide neurons, and stimulating proopiomelanocortin and cocaine and amphetamine-regulated transcript neurons, which decrease or increase, respectively, PVN pro-TRH expression (1–3, 10, 11). However, 2 weeks of caloric restriction did not reduce serum leptin or WAT mass; high levels of serum corticosterone could inhibit pro-TRH expression, TRH, and TSH release (65, 66). This is further supported by the negative associations between serum corticosterone concentration and PVN pro-TRH mRNA or serum TSH levels reported after acute exposure to several paradigms (16, 17) and detected in the restraint and the PF groups. In contrast, the decrease in serum leptin levels during exercise could explain the diminished expression of PVN pro-TRH and of serum TSH.

As early as after 1 week of voluntary WR, PVN pro-TRH expression decreased, before TSH serum concentration did, whereas MBH TRH content was not affected. This suggests that either pro-TRH precursor processing (3) was enhanced by exercise, or that TRH release from the median eminence was inhibited (31). Inhibition of TRH release from the median eminence is consistent with the late fall in TSH serum concentration, not paralleled by changes in TSH-β or D2 expression in the anterior pituitary, and with the absence of counterregulatory changes in PPII expression at the MBH level, which could influence the amount of TRH reaching thyrotropes. The increased expression of TRH-R1 in the pituitary, which is down-regulated by TRH (7), gives further support to a diminished TRH drive during steady-state conditions. Although measurement of pro-TRH expression hours after the major period of exercise may not reflect a steady state of "activity" of TRHergic neurons, results were similar in animals euthanized 3 hours after the beginning of their active phase, which suggests that 2 weeks of exercise produces a stable down-regulation of TRH neurons activity, independently of recent physical activity bouts.

Tissue or serum concentrations of THs are modulated by the activity of deiodinases (4, 11, 68). D2 is regulated in tanycytes by glucocorticoids, leptin, and THs, and in BAT by adrenergic stimulation and bile acids, whereas D1 is regulated by corticosteroids (4, 43, 67, 68). As published, food restriction diminished T_3 serum levels and the activity of hepatic D1 and BAT D2 (63), results consistent with the role of these organs in establishing circulating T_3 levels. D2 mRNA levels increased after exercise in the MBH, or in BAT probably due to increased adrenergic stimulation (69, 70). In contrast, D2 activity showed a barely significant increase in MBH, and was not significant in BAT; the high variability could stem from the transient effect of exercise-induced adrenergic stimulation and from posttranscriptional regulation because D2 has a short half-life; T_4 rapidly produces a fast ubiquitination-induced proteosomal degradation of D2 (67). Changes in D2 expression and activity of BAT from exercised rats were similar at both times of death whereas in MBH, activity increased only in animals euthanized after light onset; because we did not measure mRNA levels in Exα, we cannot confirm whether unchanged D2 activity after only 3 hours of exercise represents a delayed or an altered circadian response (71). Whether changes in D2 activity do not increase T_3 in the MBH enough to modulate PPII expression or activity (8, 9), or whether they do change but at different times, remains to be studied.

BAT is an important participant in energy expenditure (55, 70). THs, together with sympathetic activity, control nonshivering thermogenesis; upon exposure to cold, BAT is hypertrophied and lipogenesis and the activity of D2, UCP-1, and β-oxidation increase (72). BAT weight decreased after restraint but more so after exercise, proportional to number of revolutions, and in parallel with reduced size of fat droplets reflecting increased lipolytic activity. Only in sedentary and exercised rats, was increased lipid degradation proportional to PVN pro-TRH mRNA and MBH TRH levels, supporting exercise-induced concerted changes between HPT activity and energy expenditure. BAT lipolysis fuels stress-induced changes in temperature produced during the restraint episodes (50). Although we did not measure UCP-1 mRNA levels in BAT after restraint, thermogenic situations such as immobilization or overfeeding increase its expression (55, 73, 74) whereas caloric restriction diminishes it (these results and Reference 75). The lack of effect of treadmill or WR on UCP-1 mRNA levels (these results and References 75 and 76), together with the reported decrease in UCP-dependent proton conductance, supports lowered BAT thermogenic activity after exercise (77). We detected increased BAT D2 mRNA levels but not its activity, although an increased activity was reported in swim-trained female rats (78). T_3 exerts lipolytic and lipogenic effects on several tissues including BAT (79–81); voluntary exercise may blunt the effect of sympathetic stimulation on T_3 induction of lipogenic pathway, and favors lipolysis. The differential response of BAT to cold or exercise supports the proposal that its metabolic activities lie beyond thermogenesis, making BAT a potential target for weight loss (55, 70).

In contrast to the PF group, it is unlikely that the exercised group was in a state of energy deficit, because increased lipolysis in WAT tissue (5, 27, 46, 47) provides the metabolic water and energy substrates that compensate for reduced water and food intake (5, 58, 59). Inhibition of the central arm of the HPT axis by 1–2 weeks of exercise contrasted with the positive correlations between levels of

PVN pro-TRH mRNA, MBH TRH, serum TSH, or TH and number of revolutions, supporting a positive relationship between exercise and HPT activity as reported after acute increased physical activity (16, 18). Similarly, the negative correlations between WAT weight and either T_3 serum concentration, PVN pro-TRH mRNA levels, or wheel turns support a close relationship between exercise, HPT axis activity, and lipolysis; this coincides with the important role of THs in mediating catecholamine-induced lipolysis, which is increased by exercise (48, 59, 79). It is thus evident that voluntary exercise counteracts changes observed during energy deficit or excess (47, 59). In exercised, but not in PF rats, despite the reduction in the TRH drive, which should reduce TSH bioactivity (82), the decreased plasma clearance rate of T_4 (83) may maintain steady-state levels of circulating TH.

The exercise-inhibitory effect on serum TH concentrations, reported in either animals or humans, may be exclusive of conditions of energy deficit or temporal imbalance (extenuating or forced exercise, endurance training of lean subjects, exercising at high temperatures (28, 76, 84, 85)). An additional consideration is that moderate exercise (>60% VO_2) preferentially uses lipids as a fuel whereas, at higher exercise intensity, fatty acid oxidation decreases and energy is supplied by a high rate of glycolysis and protein degradation (69, 70). Intermittent running, probably reduced fatigue, overheating, and favored lipolysis, as further evidenced by the decreased serum triglycerides detected in exercised rats (present results and Reference 55). Maintenance of steady-state levels of THs would facilitate the distribution of mobilized lipids into oxidative tissues (5, 59).

Various candidate molecules may preferentially be affected by exercise and modulate pro-TRH mRNA or serum TSH levels. WR inhibits pro-opio melanocortin expression slightly (57), which could partially diminish TRH expression although neuropeptide Y expression increases only in the PF group (47, 57). Some of the molecules released by WAT during exercise do not appear to explain the changes in pro-TRH expression. Adiponectin, the serum concentration of which rises with exercise, affects preautonomic PVN pro-TRH neurons but not the hypophysiotropic ones (1, 86). IL-6 knockout has no effect on pro-TRH expression in adult mice (85–87). PVN pro-TRH neurons receive multiple afferents from extrahypothalamic regions, such as norepinephrine inputs from the brain stem, mediators of cold effect on TRH transcription (14, 15, 89, 90), γ-aminobutyric acid, or galanin inputs (91, 92); they may be targets of the brain circuitry activated by WR (35). WR attenuates stress responses in several paradigms of rodent behavior, affecting *c*-fos expression or turnover of several neurotransmitters in regions involved in stress circuitry (35); exercise may enhance galanin signaling, constraining norepinephrine output onto CRH and other stress-responsive neurons. This could be extended to PVN pro-TRH neurons and account for diminished PVN pro-TRH mRNA levels.

The DMH is part of the core-feeding circuit (93); its activation may also lead to autonomic and motor responses associated with exercise (56). We observed that WR, but not PF, increased DMH pro-CRH expression, in agreement with previous data that indicate that it contributes to reduce food intake during WR (57). We additionally observed increased levels of pro-TRH mRNA in the DMH in rats euthanized at earlier times after beginning exercise but decreased levels in PF rats. The functional circuit in which pro-TRH is expressed is unknown, but it is interesting to note that the DMH projects among other regions to the lateral hypothalamus, where TRH may exert an anorexic effect through inhibition of melanin-concentrating hormone neurons (94); this hypothetical connection may be relevant for altered feeding during WR.

In summary, decreased levels of pro-TRH expression in the PVN and of serum TSH do not necessarily alter circulating levels of THs. In contrast to the tertiary hypothyroidism produced by caloric restriction that increased serum corticosterone but not leptin levels, voluntary exercise inhibited the central arm of the HPT axis, but inhibited neither TH serum levels nor their action in target organs such as adipose tissue. This decreased expression contrasts with the increase in PVN pro-TRH expression and circulating THs that is produced by diet-induced obesity and accompanies increased leptin serum levels (74). Inhibition of PVN pro-TRH expression by exercise may relate to the lowering of serum leptin levels, and increased D2 activity in the MBH; decreased expression was accompanied by TRH accumulation at the median eminence level, suggesting decreased release of TRH coincident with low TSH serum values but not with levels of circulating THs that were normal. Because opposite changes are detected in obese overfed rats (74), changes produced by exercise may increase T_4 half-life in serum (83) and, at the same time, limit excessive release of THs in response to stimuli such as cold, circadian cycle, or feeding and contribute to the protective effects of moderate exercise on heart rate, body weight, and temperature regulation. The adaptations produced by voluntary WR might resemble those in humans with an active physical life, in which an intermittent activation of the sympatho-adrenergic system and the maintenance of serum TH levels may lead to an adequate fuel distribution and impede fat accumulation (5). These data demonstrate that voluntary WR adapts the status of the HPT axis, through pathways that are distinct

from those observed during food restriction or repeated stress.

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Address all correspondence and requests for reprints to: Patricia Joseph-Bravo, PhD, Departamento de Genética y Fisiología Molecular, Instituto de Biotecnología, UNAM, A.P. 510-3, Cuernavaca, Morelos 62271, México. E-mail: joseph@ibt.unam.mx.

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