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Research Article

Treatment with α-Methylparatyrosine Inhibits Sympathetic but Not Thyroidal Responses to Diet-Induced Thermogenesis in Lean Cafeteria-Overfed Rats

Orien L Tulp,[1,2,3]* Aftab R Awan,[1,2] George P Einstein[1,3]

[1]University of Science Arts and Technology, Montserrat, BWI

[2]University of health and Humanities Virgin Islands (Great Britain), BWI

[3]Einstein Medical Institute, Florida, USA

Abstract

The sympathoplegic drug α-methylparatyrosine (α-MPT) was administered to lean, normally fed rats or rats overfed to induce diet induced thermogenesis (DIT) with a highly palatable Cafeteria diet regimen (Café) for twelve weeks, and measures of resting thermogenesis under thermoneutral conditions (VO2 at ambient 30°C), serum T3 and 24-hour urinary vanilmandelic acid (VMA) excretion determined before and after the α-MPT treatment. The café diet resulted in significant increases in VO2, serum T3, and VMA excretion. The α-MPT resulted in approximately fifty percent decrease in the Café-induced increase in VO2 after 24hours, while thyroidal function appeared clinically unaffected. These observations suggest that the sympathetic contributions to diet-induced thermogenesis (DIT) following chronic overfeeding with the Café diet regimen represent only about half of the reported dietary induced thermic response to overfeeding and non-sympathetic contributions including the thyroidal actions that likely account for the remaining proportion of the increased DIT and sympathetic component may decrease further over time spent since feeding.

Keywords: Non-shivering thermogenesis, Sympathetic activity, α-methylparatyrosine, Rats

Introduction

Numerous reports indicate that overfeeding normally lean rats with the cafeteria feeding regimen or feeding suboptimal protein diets results in increases in resting and noradrenaline stimulated thermogenesis, termed diet induced thermogenesis (DIT) or non-shivering thermogenesis (NST) and are typically accompanied with increases in circulating triiodothyronine (T3) but not tetraiodothyronine (T4) concentrations in lean but not obese rats.[1-3] The residual effects of the acute increases in DIT have been reported to be short lived, often only lasting for a few hours in this strain of rats.[3] The outer ring (5') deiodination of T4 to the metabolically more active T3 is essential for the expression of thyroidal actions and typically occurs in man and animals under conditions of nutritional adequacy, while inner ring deiodination results in the formation of reverse T3 (rT3) under conditions of caloric deprivation including fasting, starvation, and in decreases in thermogenic demands.[4] Thus, an animal's capacity to effect changes in its rate of thermogenesis in response to alterations in diet and environment reflect its ability to modulate processes that contribute to maintaining energy homeostasis at least in part as reflections of both sympathetically and non-sympathetically mediated hormonal actions in

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***Corresponding author:** Orien L Tulp, University of Science Arts and Technology, Montserrat, USA

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multiple tissues, including skeletal muscle, liver and brown adipose tissue (BAT) vs white adipose tissue (WAT) depots and where such actions may result in energy expenditure or storage respectively.[2,4-6] During early investigations of DIT Sims suggested that there were likely physiologic similarities in the underlying mechanisms of DIT and cold-induced thermogenesis (CNST), implying that a role for brown adipose tissue might also exist for DIT as it does for CNST.[7] BAT is the only tissue of homeotherms that is recognized to have a primary function of heat generation and several investigators have now confirmed a role of BAT in the expression of DIT, which is primarily under the sympathetic control via the neurohormone norepinephrine, which can directly impinge on brown adipocyte adrenoreceptors to effect a thermogenic response.[7,8] BAT also has the capacity to deiodinate T4 to T3 in response to diet and environment, although both sympathetic and thyroidal components are necessary for the expression of DIT the relative thermogenic contributions of each physiologic participant during alterations in dietary or environmentally mediated stimuli remain unclear.[8-11]

The acute responses to thermic stimuli are well established to be modulated by norepinephrine, secreted and released via the SNS component of the autonomic nervous system (ANS) in response to nutritional and environmental stimuli where they may contribute to actions as an energy buffer.[8] Thus, the BAT mediated responses enable an organism to quickly adapt to nutritional and environmental changes and elicit a rapid response to meet short term thermogenic demands of diet and environment when required to maintain energy homeostasis.[8] The resting metabolic rats (RMR) of Café fed rats has been shown to remain elevated in the overnight or short-term fasted state however and suggest that some more longer lasting increases in basal metabolic rate (BMR) and in net energy expenditure may have occurred in response to non-SNS actions and which may remain more constant during the hours following the immediate post prandial phase of metabolism.[2, 5,9] In normal metabolism, the thyroid hormones have been attributed to account for approximately 45% of the BMR, due to ATP dependent actions on many tissues. The longer acting components may be secondary to chronically increases in SNS activity, to increases in thyroidal activity, or some combination of both factors.[5,9] In DIT, the combined effects of these two primary components of energy expenditure could readily facilitate the dissipation of excess energy as heat, thus serving as an effective buffering system of energy balance during both acute and chronic perturbations in energy intake or to conserve energy when inactive.[2,5,8,9]

The drug α-MPT can chemically ablate acute SNS activity by inhibiting the neurologic biosynthesis of catecholamine neurohormones in the CNS and peripheral neural nervous system (PNS) via inhabitation of tyrosine hydroxylase, the rate limiting enzyme of catecholamine neurotransmitter synthesis.[11-15] This agent has been shown to result in 86 to 90% reduction in catecholamine excretion within the first 24 hours following a saturating dose of the drug and which inhibitory effects persist for 32 to 40 hours or more after treatment. Accordingly, because of the α-MPT ability to virtually ablate SNS activity in rodents, it has been utilized to estimate catecholamine turnover in neural tissues following nutritional, environmental, or other stimuli. Thus, the use of α-MPT is well suited for the qualitative separation of the SNS and thyroidal components 0f DIT and NST, especially since it has long to been known to be without any effects on iodothyronine metabolism in rats or humans, in contrast to insulin and nutritional factors, both of which are linked to iodothyronine deiodination.[16-19] Thus, the purpose of the present study was to determine the relative contributions of the SNS and non-SNS components of NST in lean rats fed a low glycemic chow diet or chronically overfed with a Café diet feeding regimen. The lean phenotype of the congenic LA/Ntul//-*cp* rat strain typically remains quite lean throughout its life span, and its thermogenic responses to alterations in diet and environmental stimuli including the effects on specific isoforms of the deiodinase enzymes have been demonstrated to respond favorably and in a predictable manner.[2,20,21]

Materials and Methods

Eighteen young male littermate LA/Ntul//-cp rats were obtained from the Drexel colony and maintained under standard laboratory conditions of 22±1°C and 50% RH in plexiglass shoebox cages layered with one inch of pine shavings, and free access to house water and Purina chow #5054 from weaning. At 10 weeks of age rats were randomly divided into three equal groups and fed the Purina diet *ad libitum* (chow) or the chow diet plus a highly [palatable rendition of the cafeteria diet plus a 10% sucrose supplement in the drinking water (Café) thereafter until 24 weeks of age. A third group was continued on the chow diet to serve as a sham treatment group. Body weights and relative health of animals were monitored throughout the study. At 24 weeks of age, measures of resting thermogenesis were obtained in a Collins small animal thermogenesis apparatus, fitted with a 4 L plexiglass chamber with a closed circuit air system and the rate of oxygen utilization determined with a one liter spirometer for up to 45 minutes while maintained at thermal neutrality (30°C) in quietly resting animals and computed as ml O2/min/kg BW^{-75} as outlined by Kleiber and Wang to adjust for differences in surface area.[23] as performed in our laboratory for many years. One group of animals were administered a saturating dose of α-MPT (DL methyl para tyrosine methyl ester, 1.03mM/kg BW, i.p. Sigma chemical company) or a sham injection of an equal volume of 0.154 M NaCl solution or no injection at all (control), The treatments were administered immediately after the first recorded measure of RMR and exactly 24 hours later under the same environmental conditions. All animals were permitted

free access to their respective diets until 8 hours prior to their measurements of RMR. Colonic temperatures were obtained with a YSI rapid response thermistor inserted 12cm intracelomically before and 24 hours after the α-MPT administration to determine potential changes in body temperature due to the drug actions on SNS activity. Differences in body temperature were corrected as outlined by Kaplan and Leville to compensate for potential α-MPT induced decreases in thermoregulation. Urine was collected in replicate for 24-hour periods during the final week of the study for determination of urinary vanilmandelic acid (VMA) and at the end of the study, blood was obtained for measures of serum T3 concentration via solid phase radioimmunoassay. Data were analyzed by ANOVA with Student-Neuman-Keuls subset analysis and via students T test for unpaired data where applicable via standard statistical procedures. All three group of animals were maintained in the same animal housing room in adjacent cages thereby experiencing the same environmental temperatures throughout. The study was reviewed approved by the USAT Bioethics, Animal Care and Use Committee 172003-16-451 on Sept 14th, 2016.

Results

The body weights of all treatment groups were similar at the start of the study but by at the end of the study the Café groups had gained more weight and were significantly heavier than the control or sham groups (P=< 0.05, Figure 1. The results of control vs Café diet on urinary VMA and serum T3 are depicted in Figure 2A&2B and show that the Café diet resulted in significant elevations in both parameters (p=<0.01), consistent with other previous short-term studies of feeding a Café dietary regimen of protein restricted but calorically adequate diet.

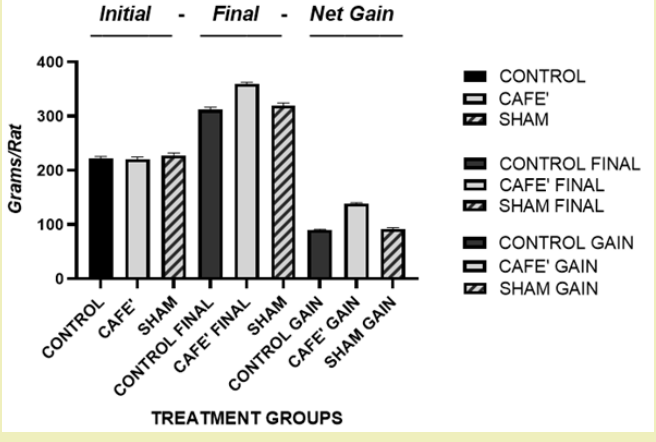

Figure 1: Body weights and weight gain of rats. Data are mean ±1 SEM, n=6 rats per group. Final vs Initial weights p=<0.05, and net weight gain of Café vs control or sham p=<0.05.

The α-MPT treatment resulted in a modest decrease in colonic temperature in both normally chow fed rats and, in the Café, fed rats, while the body temperatures of the sham treated group were unaffected by the NaCl treatment Figure 3. The mean percent decrease in colonic temperature of the α-MPT treated group was -0.52±0.03°C, and -1.39±0.34°C in the α-MPT treated controls, while the mean temperature change in the sham group averaged +0.13±0.08°C (p=<0.05 for α-MPT treatment).

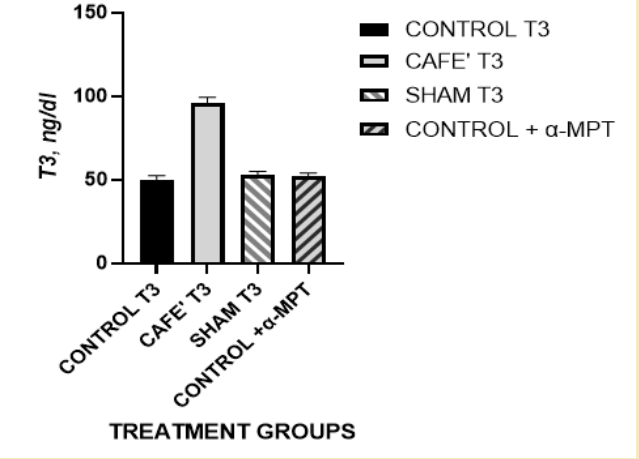

Figure 2A: Serum T3 of rats. Data are mean ±1 SEM, n=6 rats per group. Café vs all other groups p=<0.05.

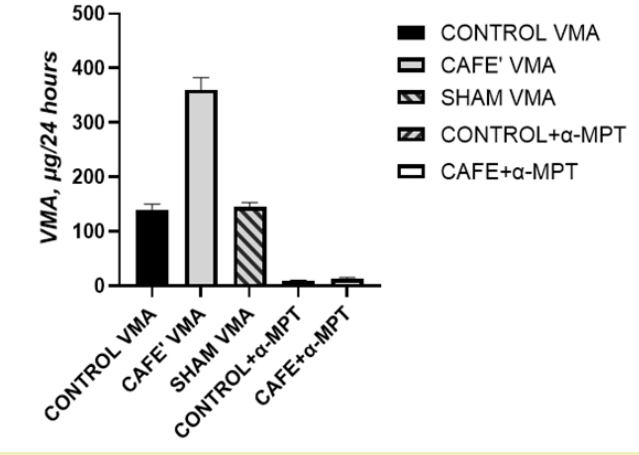

Figure 2B: Urinary Vanilmandelic acid (VMA) of rats. Data are mean ±1 SEM, n=6 rats per group. Café vs Control or sham p=<0.05.

The results of α-MPT on VO2 are depicted in Figure 4A and show that the mean increase in RMR in Café was +25% compared to normally chow-fed rats and were similar in the control and the sham treated group. (p=<0.05). The average RMR of the control group decreased by approximately 14% and in the α-MPT treated cafeteria group by an average of 16% 24 hours after the drug treatment, but the RMR remained unchanged in the sham group following the NaCl treatment. When the data were corrected via the temperature correction factors of Kaplan and Leville the data indicates that the α-MPT induced decrease in RMR averaged only 3.3% in the drug treated controls and nearly 12% in the α-MPT treated Café group and was unchanged in the sham group Figure 4B). Thus,

the α-MPT-mediated decrease in the thermally corrected RMR data remained significant only in the Café diet group.

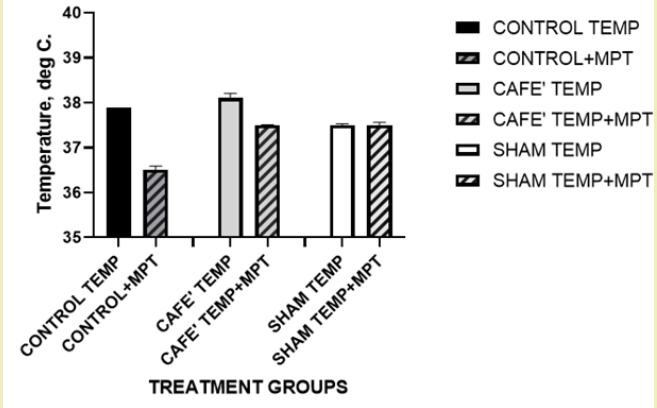

Figure 3: Effect of a-MPT on colonic temperature of rats. Data are mean ±1 SEM, n=6 rats per group. Control vs Café p+<0.05; Sham vs Sham vs. MPT=n.s.

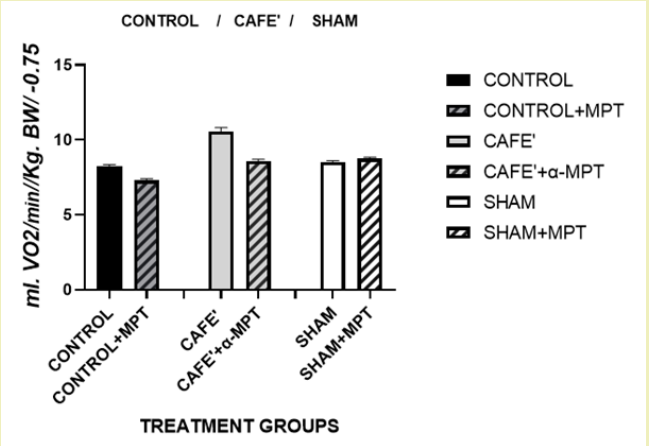

Figure 4A: Effect of a-MPT on VO2 of rats. Data are mean ±1 SEM, n=6 rats per group. Control vs Control+MPT=< 0.05; Cage vs Café +MPT = < 0.05. Sham vs Sham +MPT=n.s.

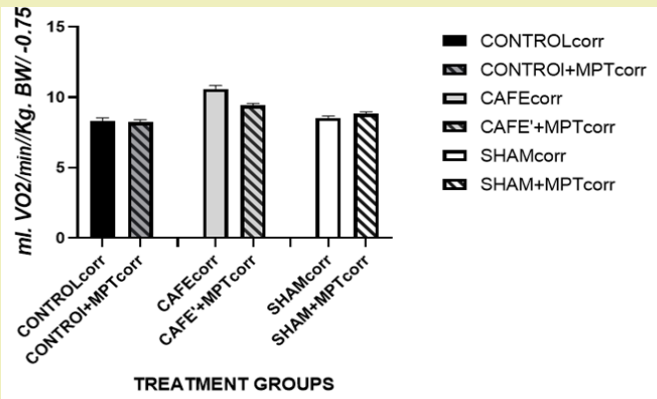

Figure 4B: Effect of a-MPT on colonic temperature of rats with thermal correction. Data are mean ±1 SEM, n=6 rats per group. Control vs Control+MPT and Sham vs Sham+MPT=n.s.: Café vs Café+MPT corrected p=<0.05.

Discussion

These results confirm that chronic, long-term feeding of a Café diet regimen to groups of normally lean rats with no know predisposition for obesity, metabolic syndrome or NIDDM results in significant increases in weight gain, circulating T3, VMA excretion, and resting VO2 when compared to normally fed and similarly housed littermates. In addition, the pharmacologic ablation of significant SNS activity with the sympathoplegic drug α-MPT resulted in significant but incomplete decreases in RMR and in colonic temperature in the Café fed rats. The effects on VO2 occurred before and after a correction for drug associated differences in colonic temperature were computed via the established Kaplan and Leville criterion and expressed relative to their body surface area.[22-23] Thus, although the results with respect to the resting VO2 in this study are qualitatively similar to the results reported by other authors, and clearly demonstrate SNS-mediated increases in the rates of DIT when rats were offered a Café' diet which reliably resulted in caloric over nutrition. The failure of α-MPT to more completely account for the magnitude of increase in DIT in the Café group suggests that thermogenic components of non-SNS origin likely also contribute to the residual DIT in chronically Café fed rats of this strain, and in this study accounted for approximately only half of the net increase in thermogenesis. The resting VO2 in the Café group averaged approximately 25% greater resting VO2 occurred than in normally fed rats, but remained only 12-14% greater after substantially complete catecholaminergic ablation of the SNS activity, and which increase remained at a similar magnitude after consideration of α-MPT linked differences in colonic temperature and surface area were factored in.

Hormonally mediated actions of insulin and thyroid hormones are believed to have contributed to the non-SNS components of DIT in this study. Both hormones are known to be required for the expression of DIT, and in their absence, diet induced increases in thermogenesis mechanisms were impaired.[6,17,24] Insulin is required for glucose uptake in skeletal muscle and adipose tissue including BAT, and in its absence or in the presence of insulin resistance, BAT thermogenesis was found to be significantly impaired.[5] Several authors have reported that glucose was the most effective macronutrient source in stimulating thermic responses to diet, although in earlier studies in young Sprague Dawley rats fed protein restricted diets, balancing the macronutrient distribution with fat was also effective in increasing circulating T3 concentrations and thermogenesis.[25,26] The significance of extrathyroidal conversion of T4 to T3 in response to alterations in diet and environment is important as it generates the active T3 hormone in close proximity to the nuclear receptors where it may bring about its intended actions, while the excess T3 generated may freely exit the cell to enter the peripheral circulation.

While decreases in insulin sensitivity were not investigated in the present study and may not have contributed substantially, the thyroidal responses are deemed to have contributed to a considerable proportion of the DIT response. BAT plays an active role in the deiodination of T4 to T3 via environmental adaptation of the extrathyroidal 5'-outer ring deiodinase activity by both differential expression of Type I (D1) and Type II (D2) deiodinases, thereby generating T3 availability for both intracellular and peripheral use has been associated with insulin sensitivity. The onset of thyroid hormone actions occurs more gradually and may persist over longer durations than catecholaminergic responses. Thyroid hormones enter tissues passively in proportion to extracellular free hormone concentrations, and subsequently bind to nuclear receptors where they promote a broad array of slower onset but longer acting actions than cell surface mediated catecholamine hormones. While catecholamine hormones are considered to elicit rapid responses to changing dietary and environmental conditions, resulting in effects that may last for minutes or hours, thyroidal and other hormones that bind to nuclear receptor regions facilitate gene transcription and are considered to elicit slower responses, often activating metabolic responses that may last for hours to days. Among the recognized effects of thyroid hormones upon activation are their effects on determining the rates of protein turnover, which consume up to 4 high energy phosphate bonds for each peptide bond formed, thereby representing one of the energetically most expensive cellular processes of intracellular metabolism, where they are largely independent of SNS effects. The nutritionally induced increases in circulating T3 concentration have their onset early within the first week of dietary exposure and have been shown to remain elevated for a duration of at least 33weeks In lean rats when the dietary intervention is prolonged. Sundin has proposed that the combined SNS and thyroidal responses contribute to the total thermogenic response of an animal, and may therefore compensate for each other in a reciprocal manner.

The Interscapular BAT (IBAT) depot is among the most highly studied of the various BAT depots in the rat, and IBAT mass and cellularity were shown to increase progressively when lean rats were overfed with a Café regimen for up to 4months post weaning, In the present study rats were overfed for a similar duration to enable optimal expression of both thyroidal and SNS thermogenic compartments thereby enabling a more accurate assessment of the relative proportions of each contributor to net energy balance. With optimal development of each of the thermogenic compartments, the relative proportions of each may be more readily quantified using the α-MPT pharmacologic probe because of their greater mass and metabolic impact of their respective compartments following the chronically-fed Café diet regimen. The current Café regimen was deemed adequate in dietary protein availability, as the nutritionally optimal Purina chow diet was freely available through the study, and all animals were observed to consume both Café and Chow diets on any given day. Despite the long duration of availability of the Café regimen, those animals were observed to gain considerably less excess weight than when the same regimen was offered to the obese phenotype of this strain.[26-29]

Thus, the present studies demonstrate the presence of multiple components in the robust expression and development of DIT commencing during the post-weaning phase of growth in the lean LA/Ntul//-*cp* rat. The contribution of BAT to the DIT responses may be envisioned to represent an acute response to the diet and to facilitate the efficient expenditure of excess or unneeded dietary energy following individual meal episodes, while minimizing the magnitude of excess energy deposition in normally lean rats. However, when demands on its thermogenic capacity persist over a more prolonged duration of nutritional or environmental challenge, the BAT may undergo additional growth and maturation, including additional hyperplasia which will likely enable the animal to enhance its net thermogenic capacity over a longer duration, as once the BAT cells proliferate, they likely remain present and potentially active thereafter assuming that no metabolic, hormonal, environmental or pharmacologic agents have intervened to compromise their activity.[28] While the specific role of the nutritionally induced increases in circulating T3 in the present study are only speculative, they may in addition to other broad-spectrum actions on overall metabolism, may also contribute to the continued hyperplasia of the BAT depots through the immediate post weaning and preadolescent growth phases. Regardless of the physiologic mechanisms, the non-SNS components appeared to contribute significantly to the total thermogenic response in this study, as elucidated following the virtual depletion of α-MPT -mediated effects on SNS controlled aspects of thermogenic activity during overfeeding with the Café diet regimen. In contrast, the SNS-contribution was of only modest proportions in normally fed lean rats where mechanisms of appetite and energy homeostasis likely remained unperturbed.

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None.

Conflicts of Interest

Author declares that there is no conflict of interest.

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