A high-fat diet impairs cooling-evoked brown adipose tissue activation via a vagal afferent mechanism

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Madden CJ, Morrison SF. A high-fat diet impairs cooling-evoked brown adipose tissue activation via a vagal afferent mechanism. Am J Physiol Endocrinol Metab 311: E287–E292, 2016. First published June 28, 2016; doi:10.1152/ajpendo.00081.2016.—In dramatic contrast to rats on a control diet, rats maintained on a high-fat diet (HFD) failed to activate brown adipose tissue (BAT) during cooling despite robust increases in their BAT activity following direct activation of their BAT sympathetic premotor neurons in the raphe pallidus. Cervical vagotomy or blockade of glutamate receptors in the nucleus of the tractus solitarius (NTS) reversed the HFD-induced inhibition of cold-evoked BAT activity. Thus, a HFD does not prevent rats from mounting a robust, centrally driven BAT thermogenesis; however, a HFD does alter a vagal afferent input to NTS neurons, thereby preventing the normal activation of BAT thermogenesis to cooling. These results, paralleling the absence of cooling-evoked glucose uptake in the BAT of obese humans, reveal a neural mechanism through which consumption of a HFD contributes to reduced energy expenditure and thus to weight gain.
Initially, both TCORE and TSKIN were maintained >36.0°C with a heat lamp and perfusion of the thermal blanket with warm water. Rats were cooled by turning off the heat lamp and perfusing the thermal blanket with cool water to reach a target skin temperature of 36.0 to 35.0°C. During the skin cooling, BAT SNA, TBAT, TCORE, and expired CO2 were considered as dependent variables and were allowed to change freely. Using Spike 2 software (CED), a continuous measurement (4-s bins) of BAT SNA amplitude was obtained as the root mean square value of the BAT SNA (square root of the total power in the 0.1 to 20 Hz band) from the autospectra of sequential 4-s segments of BAT SNA. The baseline level of BAT SNA was taken as the mean BAT SNA amplitude during a 2-min period of minimum BAT SNA recorded when the rat was in a warm condition (TCORE and TSKIN >35.5°C) and basal BAT SNA was absent. For the cooling response, statistical comparisons were made between groups, using the values of the individual variables over the 30-s period when the TSKIN reached a nadir of 35.5 ± 0.5°C. In several rats maintained on a control diet, a TSKIN of 35.5°C was not attained, since the robust activation of cooling-evoked BAT thermogenesis counteracted the fall in TSKIN. After the target TSKIN was attained, a subset of the rats maintained on a HFD (n = 5) received bilateral nanoinjections of a glutamate receptor antagonist (kynurenate, 80 nl, 0.1 M, or a combination of AP5/CNQX 80nl, 5:5 mM) into the NTS (coordinates from calamus scriptorius: 1.2 mm rostral, 1.0 mm lateral, and 0.6 mm ventral). Another subset of rats maintained on a HFD (n = 5) received either bilateral nanoinjections of vehicle (saline, 60 nl; n = 2) or no nanoinjection (n = 3). The responses in the vehicle-injected control rats and the rats receiving no injection were similar, so these rats were considered a single group for statistical comparisons. Statistical comparisons were made between the 30-s period just prior to the NTS nanoinjections and the peak 30-s values of the individual variables within 30 min of the nanoinjection or for controls receiving no injection within 30 min of reaching the target TSKIN of 35.5°C. In another group (n = 5) of HFD rats, bilateral cervical vagotomies were performed at the time of tracheal cannulation prior to undergoing the protocol and cooling procedures identical to all other vagus-intact rats. To section the cervical vagi, a midline incision was made along the ventral surface of the neck, and the muscles overlaying the common carotid arteries were separated with blunt dissection to expose the cervical vagi. Subsequently, an ~4-mm portion of each cervical vagus was dissected from each common carotid artery in the region just rostral to the clavicle. A suture was placed around each vagus nerve, and after slight retraction, the vagi were sectioned completely using angled iridectomy scissors, and ~2 mm of vagus nerve was then sectioned from the proximal end of the sectioned vagus. In every case, this simple procedure produced a complete section of the cervical vagi that was easily verified visually as well as by the marked slowing in respiratory rate and increase in heart rate following the section of the second vagus. For the responses evoked by nanoinjection of the GABA_A antagonist bicuculline (Bic) in the rostral raphe pallidus area (rRPa; coordinates from calamus scriptorius: 2.8–3.0 mm rostral on the midline, 3.0 mm ventral), statistical comparisons were made between groups using the difference between the 30-s period just prior to the injection and the peak 30-s values of the individual variables within 15 min of the injection.

All statistics were performed using Systat Software (version 10; Cranes Software International). Data are expressed as means ± SE. Statistical significance was assessed using a paired t-Test or ANOVA. Post hoc comparisons were performed using layered Bonferroni corrections. Results with P < 0.05 were considered significant.

**RESULTS**

To test the hypothesis that cooling-evoked increases in BAT SNA and BAT thermogenesis are impaired in rats maintained on a HFD compared with rats maintained on a control diet, BAT SNA and TBAT were measured during skin cooling. Since activation of vagal afferent nerve fibers can inhibit BAT SNA (17) and vagal afferent activity is responsive to dietary composition (22, 28), we also determined whether vagal afferent...
ent activity is required for a HFD-induced impairment of cooling-evoked BAT SNA and BAT thermogenesis. In rats maintained on the control diet, skin cooling increased BAT SNA dramatically \((+828 \pm 188\% \text{ of baseline}, n = 7, P < 0.01; \text{Fig. 1A})\). In sharp contrast, rats maintained on a HFD and with intact vagus nerves failed to increase their BAT SNA \((+2 \pm 2\% \text{ of baseline}, n = 19; \text{Fig. 1B})\) to skin cooling. However, in rats that were maintained on a HFD but acutely vagotomized immediately prior to BAT SNA recordings, skin cooling elicited a robust increase in BAT SNA \((+519 \pm 78\% \text{ of baseline}, n = 5, P < 0.01; \text{Fig. 1C})\). The TSKIN attained by circulating cool water through a cooling blanket surrounding the rat’s trunk was not significantly different among groups \((\text{control diet}: 35.9 \pm 0.2°C; \text{HFD}: 35.5 \pm 0.1°C; \text{HFD with vagotomy}: 35.5 \pm 0.1°C)\). As expected, TBAT initially decreased with skin cooling in all groups; however, the sympathetic activation of BAT thermogenesis that occurred in the control diet and in the vagotomized HFD groups resulted in levels of TBAT that were significantly higher than that in the vagus intact HFD group \((35.5 \pm 0.2°C \text{ vs. } 32.7 \pm 0.2°C, P < 0.001)\).

During skin cooling, TCORE decreased in all groups but was sustained at a higher level in the control diet group compared with the HFD group \((36.1 \pm 0.2°C \text{ vs. } 35.4 \pm 0.1°C, \text{respectively}, P < 0.01)\), which was likely due to differences in BAT thermogenesis. The TCORE of the vagotomized HFD rats \((35.6 \pm 0.2°C)\) did not differ from that in either the control diet or the HFD rats \((P = 0.078 \text{ and } P = 0.433, \text{respectively})\).

Expired CO\(_2\) during cooling did not differ between the control diet and HFD groups \((\text{control diet}: 4.4 \pm 0.2\% \text{ vs. } \text{HFD: } 4.1 \pm 0.2\%, \text{HFD vagotomized: } 4.4 \pm 0.4\%)\).

In rats maintained on a HFD and with intact vagi, bilateral nanoinjections \((60 \text{ nl})\) of the glutamate receptor antagonists \((2R)-\text{amino-5-phosphonovaleric acid (AP5; } 5 \text{ mM})\) and \(6\text{-cyano-7-nitroquinoxaline-2,3-dione (CNQX; } 5 \text{ mM})\) or kynurenate into the NTS \((\text{Fig. 2C})\) restored the activation of BAT SNA and TBAT to skin cooling \((\text{Fig. 2, A and B})\). As shown at the beginning of the traces in Fig. 2, A–C, and in Fig. 10 min
The Bic-evoked increase in expired CO$_2$ was significantly greater in the control diet (3.0% ± 0.1% in control diet vs. 0.4% ± 0.2% in HFD, $P < 0.01$; Fig. 3, A and B). In contrast, TBAT continued to fall from 32.5 ± 0.2°C prior to the nanoinjections of saline and glutamate receptor antagonists into the NTS to 31.5 ± 0.3°C ($P < 0.01$, n = 5) 30 min after these nanoinjections (Fig. 2C).

To assess a potential difference in the functionality of the neural pathway between the BAT sympathetic premotor neurons (17) in the rRPa and the interasculapar BAT in rats maintained on a HFD, the GABA$_A$ receptor antagonist Bic was nanoinjected in the rRPa (Fig. 3, C and D) of rats maintained on control diet ($n = 7$) and rats maintained on HFD ($n = 5$). Bic in the rRPa increased BAT SNA in both groups, with the increase being larger in rats on the control diet (+1.409 ± 144% of baseline in rats on control diet compared with +481 ± 105% of baseline in rats on HFD, $P < 0.05$; Fig. 3, A and B). The increases in TBAT evoked by Bic in the rRPa were not different between rats on the control diet (3.0 ± 0.5°C) and those on HFD (3.5 ± 0.4°C). The Bic-evoked increase in expired CO$_2$ was significantly different between groups (+1.0 ± 0.1% in control diet rats vs. +1.3 ± 0.1% in HFD rats, $P < 0.05$).

**DISCUSSION**

Since the ambient temperature in which rats are maintained is normally below their thermoneutral zone, their cooling-evoked activation of BAT makes a tonic contribution to their overall energy expenditure. The major finding of this study is that maintenance on a HFD markedly reduces the cooling-evoked increases in BAT SNA and BAT thermogenesis observed in rats on a control diet. This reduction in BAT activity is dependent on the glutamatergic excitation of NTS neurons by a population of vagal afferents. Thus, consumption of a HFD enhances a vagal afferent mechanism that chronically reduces BAT energy expenditure and thereby contributes to HFD obesity. The consistent finding that the cooling-evoked activation of BAT is markedly diminished in obese humans (5, 20, 24, 31) raises the striking possibility that this parallel reduction in human BAT energy expenditure also arises from an enhanced vagal afferent mechanism due to exposure to a HFD.

Vagal nerve section or blockade of glutamate receptors in the NTS reversed the HFD-induced inhibition of cooling-evoked increases in BAT SNA and BAT thermogenesis, implicating an active glutamatergic excitation of a population of second-order vagal viscerosensory NTS neurons in the central neural circuit mechanism underlying the HFD-induced abrogation of cooling-evoked BAT thermogenesis. The NTS is the termination site of vagal afferents, and glutamate is their primary neurotransmitter (2), and activation of neurons in the NTS produces an inhibition of BAT SNA and BAT thermogenesis (4, 30). Diverse populations of vagal afferents can influence the regulation BAT; both activation and inhibition of BAT SNA can be evoked by electrical stimulation of vagal afferents (17). Short-term consumption of a HFD increases sympathetic activation of BAT (11) likely via vagal afferents that are activated by lipid in the duodenum (3). In contrast, long-term maintenance on a HFD, a model reflecting the state of many in the obese human population, results in impaired BAT activity (current study and Ref. 10), which is likely due to vagal activation of BAT sympathoinhibitory neurons in NTS (4, 30).

HFD could alter either the level of discharge of the relevant vagal afferent and/or the responsiveness of its NTS target neurons, including via a potential glial modulation of glutama-

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**Fig. 3.** Blockade of GABA$_A$ receptors in the rostral raphe pallidus area (rRPa) increases BAT activity in all rats whether maintained on a control diet or on a HFD. A and B: nanoinjection of the GABA$_A$ receptor antagonist bicuculline (Bic) in the rRPa increases BAT SNA, TBAT, and expired CO$_2$ in a rat maintained on a control diet (A) and in a rat maintained on a HFD (B). C: histological coronal section illustrating an injection site in the rRPa. D: a composite mapping of the centers of the rRPa injection sites in 12 rats plotted on an atlas drawing through the rat rostral medulla (21).

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**A** Control Diet  
Bic rRPa

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Control Diet

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High Fat Diet

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Bic rRPa

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Control Diet

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High Fat Diet

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ergic transmission in NTS (32) or via an augmented glutamate release from vagal afferent terminals due to lipid modulation of their TRPVP1 conductance (1). The relevant population of vagal afferents required for the effects of HFD on BAT activation, as well as the nature of the stimulus to which they respond, which is potentially augmented in HFD rats, remains to be identified.

Of interest are the hepatic afferents responsible for the decrease in BAT thermogenesis following the upregulation of hepatic glucokinase during high-fat feeding (29). Another possibility is that activation of peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear receptor for dietary-derived lipids that is found in the nodose ganglion cell bodies of vagal afferents (12), drives the altered vagal afferent activity that results in an impaired BAT SNA and BAT thermogenesis. In this regard, chronic administration of PPAR receptor agonists decreases norepinephrine turnover in BAT, reduces oxygen consumption, and increases weight gain (6). Knockout of PPARγ in Phox2b-expressing vagal afferent neurons in the nodose ganglion led to elevated oxygen consumption and heat production in HFD mice (12), consistent with the potential for PPARγ activation in primary vagal sensory neurons to inhibit BAT thermogenesis. Although PPARγ mRNA levels in nodose ganglion were reduced in HFD mice (12), the elevated levels of dietary-derived lipids during exposure to a HFD may be sufficient to induce an inhibition of BAT thermogenesis, even in the face of PPARγ mRNA downregulation in nodose ganglion neurons.

The finding that blockade of GABA_A receptors in rRPa significantly increased BAT SNA, independent of diet, indicates that a HFD does not eliminate all nonthermoregulatory (i.e., tonic) excitatory inputs to BAT sympathetic premotor neurons in rRPa (14), nor does it result in a sufficiently strong inhibition of BAT sympathetic preganglionic or ganglion neurons such that all excitations of BAT SNA via stimulation of BAT sympathetic premotor neurons are blocked in HFD-fed rats. However, the determination that the level of BAT SNA evoked by disinhibition of BAT sympathetic premotor neurons in rRPa was smaller in rats on a HFD compared with rats on a control diet does suggest that a HFD alters the relative levels of tonic excitation and inhibition at site(s) in the BAT sympathetic-excitatory pathway from the BAT sympathetic premotor neurons in rRPa to the BAT ganglion cells. In this regard, our data would be consistent with a scenario in which a HFD increased a tonic or vagally mediated inhibition of BAT sympathetic preganglionic or ganglion neurons to a degree that was sufficient to block the cold-evoked increase in BAT SNA but only reduced that evoked by disinhibition of neurons in rRPa. In this regard, the neural circuit mechanism through which vagal stimulation (17) or NTS neurons (4, 30) can inhibit BAT SNA has not yet been determined. Alternatively, since the quantification of SNA is strongly influenced by the contact between the nerve and the recording electrodes, the difference in BAT SNA responses to Bic in rRPa could arise from differences in the “adipose insulation” on the BAT nerve bundles between the rats on HFD and on control diet, although there were no obvious differences noted during the surgical isolation of the nerves.

Although disinhibition of neurons in rRPa in rats on a HFD evoked a smaller increase in BAT SNA than in rats on a control diet, the evoked increase in BAT thermogenesis (monitored via TBAT) was not different between rats on a HFD and those on a control diet, and the Bic-evoked increase in expired CO2 (an index of metabolism) was larger in rats on HFD than in those on control diet. These data are consistent with an enhanced effect of BAT SNA on BAT thermogenesis and on BAT energy expenditure in rats on a HFD. They also suggest that stimuli capable of overcoming the HFD-induced abrogation of cooling-evoked BAT activation would result in potentiated BAT thermogenesis, consistent with the findings that norepinephrine-evoked increases in oxygen consumption are increased by a cafeteria diet (23) and that UCP-1 expression in BAT is increased by HFD (7).

Novel mechanisms to upregulate BAT energy expenditure for obesity therapy have focused on increasing the expression of UCP-1 in adipose tissue. Although this approach would increase the metabolic capacity of adipose tissue, a major implication of the current study is that upregulation of UCP-1 in adipose tissue, as seen in diet-induced obesity (7), might not significantly increase BAT energy expenditure, since the HFD also impairs activation of BAT sympathetic outflow. A similar critical point was made by the demonstration that PPARγ receptor agonists increased UCP-1 expression in adipose tissue but failed to increase energy expenditure (19, 26). The current studies demonstrate that vagal afferent activation of neurons in the NTS is essential for the HFD-induced impairment of BAT activation. These data provide an important foundation for defining potential targets for therapeutic approaches to reverse these HFD-induced impairments and thereby increase fat metabolism in obesity.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.J.M. and S.F.M. conception and design of research; C.J.M. performed experiments; C.J.M. analyzed data; C.J.M. and S.F.M. interpreted results of experiments; C.J.M. and S.F.M. prepared figures; C.J.M. drafted manuscript; C.J.M. and S.F.M. edited and revised manuscript; C.J.M. and S.F.M. approved final version of manuscript.

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