Published in final edited form as:

Brain Res. 2019 May 01; 1710: 136–145. doi:10.1016/j.brainres.2019.01.002.

Glucoregulatory responses to hypothalamic preoptic area cooling

Kenjiro Muta^{1,4}, Miles E. Matsen¹, Nikhil K. Acharya¹, Darko Stefanovski², Richard N. Bergman³, Michael W. Schwartz¹, and Gregory J. Morton¹

¹University of Washington Medicine Diabetes Institute, Department of Medicine, University of Washington, Seattle, WA, USA ²New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA. ³Diabetes and Obesity Research Institute, Cedars-Sinai Medical Center, Los Angeles, California, USA. ⁴Current address: Chakri Naruebodindra Medical Institute, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Abstract

Normal glucose homeostasis depends on the capacity of pancreatic β -cells to adjust insulin secretion in response to a change of tissue insulin sensitivity. In cold environments, for example, the dramatic increase of insulin sensitivity required to ensure a sufficient supply of glucose to thermogenic tissues is offset by a proportionate reduction of insulin secretion, such that overall glucose tolerance is preserved. That these cold-induced changes of insulin secretion and insulin sensitivity are dependent on the sympathetic nervous system (SNS) outflow suggests a key role for thermoregulatory neurons in the hypothalamic preoptic area (POA) in this metabolic response. As these POA neurons are themselves sensitive to changes in local hypothalamic temperature, we hypothesized that direct cooling of the POA would elicit the same glucoregulatory responses that we observed during cold exposure. To test this hypothesis, we used a thermode to cool the POA area, and found that as predicted, short-term (8-h) intense POA cooling reduced glucose-stimulated insulin secretion (GSIS), yet glucose tolerance remained unchanged due to an increase of insulin sensitivity. Longer-term (24-h), more moderate POA cooling, however, failed to inhibit GSIS and improved glucose tolerance, an effect associated with hyperthermia and activation of the hypothalamic-pituitary-adrenal axis, indicative of a stress response. Taken together, these findings suggest that POA cooling is sufficient to recapitulate key glucoregulatory responses to cold exposure.

Address for correspondence: Gregory J. Morton, Department of Medicine, University of Washington at South Lake Union, 750 Republican St, F771, Box 358062, Seattle, Washington, 98195, USA. Phone: (206) 897 5292 Fax: (206) 897 5293, gjmorton@uw.edu.

Author Contributions

Conceived and designed the experiments: KM, MEM, MWS, GJM. Performed the experiments: KM MEM NKA. Analyzed the data: KM, DS, RNB, MWS, GJM. Wrote the manuscript: KM, MWS, GJM.

Declarations of interest: none

Keywords

hypothalamic preoptic area; insulin secretion; insulin sensitivity; glucose tolerance; thermoregulation

1. INTRODUCTION

Maintenance of normal glucose tolerance hinges on the capacity of pancreatic β -cells to adjust insulin secretion in response to changes of tissue insulin sensitivity (Bergman et al., 1979; Bergman et al., 1981; Kahn et al., 1993). During insulin-resistant conditions such as obesity (Buchanan et al., 1990; Kahn et al., 1993; Moran et al., 1999), for example, preservation of normal glucose tolerance requires a proportionate increase of insulin secretion while conversely, states of increased insulin sensitivity (e.g., exercise, weight loss or cold exposure) (Bukowiecki, 1989) require a compensatory reduction of insulin secretion to avert hypoglycemia. Consistent with this concept, the relationship between insulin secretion and insulin sensitivity is hyperbolic across multiple species including humans (Kahn et al., 1993), dogs (Stefanovski et al., 2011), and rats (Morton et al., 2017). Consequently, the product of the two (referred to as the Disposition Index (DI), a measure of insulin-mediated glucose disposal) tends to remain constant in normal individuals, such that glucose tolerance remains unchanged even in the face of conditions that strongly impact tissue glucose utilization. The physiological importance of this coupling process is evident in the fact that progression from normal glucose tolerance to type 2 diabetes (T2D) involves the failure to mount an appropriate beta cell response to worsening insulin resistance. Yet the mechanisms that couple insulin secretion to insulin sensitivity remain poorly understood.

Cold-exposure has proven useful as a paradigm for investigating this coupling process because thermoregulatory responses that preserve core body temperature are dependent upon rapid, marked and reciprocal changes of insulin secretion and insulin sensitivity. Specifically, maintenance of core body temperature in a cold environment depends upon increased heat production (Morrison, 2016) that in turn is coupled to marked increases of insulin sensitivity and glucose uptake into thermogenic tissues (Smith et al., 1986), with glucose tolerance remaining unchanged owing to a compensatory reduction of insulin secretion (Morton et al., 2017; Vallerand et al., 1983). Moreover, the effect of cold exposure to elicit these rapid, potent and reciprocal changes of insulin sensitivity and insulin secretion appears to depend upon increased sympathetic nervous system (SNS) outflow, since the effects are rapidly reversed by intravenous infusion of the alpha-adrenergic receptor antagonist, phentolamine (Morton et al., 2017). Taken together, these findings implicate the brain in adaptive changes of insulin secretion and insulin sensitivity that preserve normal glucose tolerance during cold exposure.

Based on these observations, we hypothesized that direct activation of hypothalamic neurocircuits responsible for these adaptive thermoregulatory responses should mimic the effect of cold exposure on insulin sensitivity and insulin secretion. These thermoregulatory neurocircuits are located in the hypothalamic preoptic area (POA), and process afferent thermal information conveyed by thermosensory receptors in the skin (Morrison et al., 2014;

Morrison, 2016). This cutaneous sensory information is then conveyed via a well-mapped peripheral-to-central afferent relay to POA neurons that in turn coordinate adaptive autonomic (brown adipose tissue (BAT) activation, shivering, vasoconstriction) (Morrison et al., 2014; Nakamura and Morrison, 2011) and behavioral responses (nesting, seeking warmth/cold) (Tan et al., 2016) that work in concert to maintain a stable core temperature. Interestingly, many of these POA neurons are themselves thermosensitive, with some described as warm-sensitive neurons (WSN) while others are cold-sensitive. During cold exposure, inhibition of POA WSNs increases SNS outflow to thermogenic tissues while reducing heat loss via both behavioral and autonomic means by activating downstream neurons in the dorsomedial nucleus (DMN) and other areas (Morrison et al., 2014; Morrison, 2016). Because at least some of these POA neurons can sense and are responsive to changes in local brain temperature (Imai-Matsumura and Nakayama, 1987), direct cooling of the POA is sufficient to mimic the effect of cold exposure to induce BAT thermogenesis, vasoconstriction and shivering (Gale et al., 1970; Hammel et al., 1960), while warming this brain area reduces energy expenditure and induces panting, sweating and cold-seeking behavior (Carlisle, 1966; Magoun et al., 1938). Accordingly, we reasoned that if the potent effect of cold exposure on insulin secretion and insulin sensitivity is coordinated by POA neurocircuits, these responses should be elicited by direct cooling of this brain area. We therefore developed and validated a water-circulating thermode with which to cool the POA of rats, and utilized this approach to determine whether cold-induced activation of POA neurocircuits is sufficient to recapitulate the effect of cold exposure on determinants of glucose tolerance (Morton et al., 2017).

2. RESULTS

2.1. Validation of hypothalamic POA cooling

To test the hypothesis that direct cooling of temperature-sensitive POA neurons is sufficient to mimic the effect of cold exposure on determinants of glucose tolerance, we developed a water-perfused thermode system designed to modulate local POA temperature (Fig. 1A) (Banet and Séguin, 1967). As a first step, we sought to confirm the effect of thermode-induced cooling or warming on local hypothalamic temperature. To accomplish this, we perfused either ice-cold, room temperature, or heated water through the thermode for 5 min and measured local hypothalamic temperature using a thermocouple placed approximately 1.0 mm lateral to the thermode tip (Fig. 1B) in anesthetized rats that were placed on a heating pad to maintain core body temperature at $\sim 35^{\circ}\text{C}$. Here, we found that by perfusing either room temperature (POA moderate cooling) or ice-cold (POA intense cooling) water through the thermode, local hypothalamic temperature declined within 5 min (by $2.2 \pm 0.4^{\circ}\text{C}$ and $7.6 \pm 0.5^{\circ}\text{C}$, respectively; $p < 0.05$) (Fig. 1C). Conversely, perfusing heated (POA warming) water through the thermode had the opposite effect, raising hypothalamic temperature by $3.5 \pm 0.4^{\circ}\text{C}$ within 5 min ($p < 0.05$) (Fig. 1C). In none of these studies was any effect on rectal temperature observed (data not shown, $p = \text{ns}$). Thus, the thermode-based system described herein is capable of rapidly and effectively inducing either local cooling or warming of the POA.

To determine the effect on core body temperature of local POA warming or cooling via the POA-implanted thermode over a longer period of time ($t=60$ min), we studied a separate cohort of conscious, unrestrained rats housed at room temperature. Consistent with their effect to reduce local POA temperature (Fig. 1C), POA cooling with either room temperature or ice-cold water elicited rapid and sustained increases of core body temperature (between $t=10$ – 60 min, Fig. 1D, $p<0.05$) that returned to normal ($t=120$ min) following cessation of perfusion ($t=60$ min). Conversely, perfusion with heated water had the opposite effect (*i.e.*, of reducing core temperature; $t=30$ – 60 min, Fig. 1D, $p<0.05$). These findings extend previous work (Imai-Matsumura and Nakayama, 1987) documenting the ability of thermode-induced POA cooling or warming to potentially engage adaptive thermoregulatory responses to cold or heat exposure, respectively. To control for the possibility that thermode implantation in and of itself has an independent effect, we also measured core body temperature in thermode implanted animals that received no perfusion relative to those not implanted with the thermode. We found no difference in core temperature (Fig. 1D, $p=ns$), body weight or food intake when comparing animals that were implanted with the thermode (but not perfused) and those that were not (Fig. 1E and F, $p=ns$).

2.2. Time course effect of cold exposure on glucose metabolism

Our recent work demonstrates that relative to those housed at room temperature, rats exposed to at 5°C for 28 h (without access to food, to avoid the confounding effects on food intake) exhibited a marked increase of insulin sensitivity, yet glucose tolerance remained unchanged owing to a precisely calibrated compensatory decrease of insulin secretion (Morton et al., 2017). As a first step to investigate the time-course over which these responses to cold exposure are mounted, we compared measurements of blood glucose and plasma insulin levels in fasted rats that were either exposed to cold (5°C) or housed at room temperature for 4, 8 or 28 h. As expected, blood glucose levels declined steadily in both groups as a result of the increased duration of fasting. Nevertheless, glucose levels were slightly lower in the cold-exposed animals, but only at the 8-h time point (Fig. 2A). In contrast, plasma insulin levels were reduced at each time point in cold-exposed rats relative to those housed at room temperature (Fig. 2B), raising the possibility that insulin sensitivity increases rapidly during cold exposure, and that whereas this effect is initially associated with a reduction of glycemia, it is compensated for over time such that by 28h, glucose values are no different between groups. Based on this time course, we therefore compared the effects of both short but intense (8 h) and long but moderate (24 h) POA cooling on determinants of glucose tolerance.

2.3. Effect of short-term, intense POA cooling on determinants of glucose tolerance

To determine if POA cooling recapitulates the glucose metabolic effects of cold exposure, we performed a frequently sampled intravenous glucose tolerance test (FSIGT) on rats subjected to either an 8-h period of intense POA cooling (by perfusion of ice-cold water at 4 ml/min) or control (no thermode perfusion) (Fig. 3A). In contrast to what was observed during cold exposure (Fig. 2), baseline blood glucose levels increased slightly (relative to controls) in response to this intervention (Fig. 3B), and this effect was associated with both a slight increase of plasma insulin levels (Fig. 3C) and a marked increase of plasma corticosterone levels (Fig. 3D). Together, these effects suggest that in addition to inducing

thermoregulatory responses normally induced by cold exposure, intense POA cooling elicits a stress response, which can potentially confound key metabolic outcomes (e.g., insulin secretion, insulin sensitivity).

Despite the potentially confounding influence of stress induced by intense POA cooling, a more detailed analysis of data obtained during the FSIGT shows a striking similarity to the metabolic response elicited by cold exposure (Morton et al., 2017). Specifically, glucose tolerance was unaffected by intense POA cooling (Glc AUC : 6332 ± 265 for no perfusion vs. 6744 ± 288 for POA cooling; $p=\text{ns}$) (Fig. 4A–C), despite a marked suppression of the insulin response to iv glucose, whether measured either after the first 5 min ($t=5\text{--}8$ min; $p<0.05$) (Fig. 4D and E) or over the first 20 min ($\text{Ins AUC}_{0-20 \text{ min}}$: 2224 ± 360 for no perfusion vs. 1357 ± 142 for POA cooling; $p<0.05$) (Fig. 4F). Interestingly, however, the AIR_G was not affected (Fig. 4G), raising the possibility that the inhibitory effect we observed involved inhibition of the 2nd phase, rather than the 1st phase of glucose-induced insulin secretion. This combination of preserved glucose tolerance despite reduced insulin secretion during POA cooling implies that either insulin sensitivity or insulin-independent glucose uptake (or both) must have increased. While we observed a trend towards elevated S_I , the effect did not achieve statistical significance, nor was S_G affected by POA cooling (Fig. 1H and J). Thus, the increase of insulin sensitivity characteristic of the response to cold was not observed during this intervention, potentially owing to the associated stress response. Collectively, these data indicate that short-term, intense POA cooling captures some, but not all, of the effects of cold exposure on whole-body glucose metabolism.

2.4. Effect of longer-term, moderate POA cooling on determinants of glucose tolerance

In an effort to mitigate the apparent stress associated with intense POA cooling, we next investigated the effect of moderate POA cooling on determinants of glucose tolerance over a longer time interval (Fig. 5A). To achieve this goal, fasted rats were perfused with room temperature water at a flow rate of 1 mL/min through the POA-implanted thermode for 24h, after which they were subjected to a FSIGT and subsequent minimal model analysis. Unlike the response to cold exposure (Morton et al., 2017), this intervention had no effect on basal plasma insulin levels, and once again, we found that blood glucose and plasma corticosterone levels were elevated relative to controls (Fig 5B–D). Despite this evidence of a stress response, glucose tolerance was improved by this intervention (Glc AUC : 7481 ± 212 for no perfusion vs. 4539 ± 247 for POA cooling; $p<0.05$) (Fig. 6A–C), most likely because of a trend towards increases of both S_I and S_G , combined with an increase of the AIR_G (in the absence of any change of overall insulin secretion during the FSIGT) (Fig. 6G, H, J). Thus, the overall effect of moderate POA cooling over a 24 h period was to improve glucose tolerance via combined increases of S_I , AIR_G and S_G . This pattern of responses is again distinct from what is observed during cold exposure.

3. DISCUSSION

The ability to maintain core body temperature during cold exposure requires a marked increase of heat production in tissues such as skeletal muscle and brown adipose tissue. To support this thermogenic response, glucose uptake into thermogenic tissues must also

increase, and yet glucose tolerance is not affected. Work from our lab and elsewhere has shown that glucose homeostasis is preserved in this setting through rapid, highly coordinated and reciprocal changes of insulin secretion and insulin sensitivity, and that these responses appear to be driven via the SNS (Morton et al., 2017). Although the primary source of afferent input relevant to thermoregulation is supplied to neurons in the POA by cutaneous thermosensory neurons, subsets of POA neurons involved in this process have been identified that are themselves thermosensitive and capable of responding to changes in local hypothalamic temperature in ways that seem to recapitulate the response to a change in ambient temperature. Consequently, POA cooling induces the same adaptive thermogenic responses observed during cold exposure (*e.g.*, BAT activation and shivering) (Gale et al., 1970; Hammel et al., 1960). In the current study, we sought to determine if the adaptive changes of insulin secretion and insulin sensitivity that preserve glucose homeostasis during cold exposure can also be recapitulated by localized POA cooling. We found that short-term (8-h), intensive POA cooling mimics the effect of cold exposure to reduce insulin secretion in the absence of any change of glucose tolerance, presumably because insulin sensitivity tended to increase such that the overall insulin effect (as measured by product of AIR_G and Si, known as the Disposition Index) did not change. In contrast, longer-term (24-h), moderate POA cooling substantially improved glucose tolerance, an effect that is not typically observed during cold exposure and was mediated by an increase in the Disposition Index. These findings show that while some of glucoregulatory responses to cold exposure are recapitulated by POA cooling, others are not. Although the basis for these differences is unknown, the findings of mildly increased basal glucose and markedly increased corticosterone levels are suggestive of a stress response. Also consistent with this hypothesis is the effect of POA cooling to induce hyperthermia, an effect that is not observed during cold exposure.

Neurons in the hypothalamic POA integrate and respond to temperature information supplied by temperature-sensitive neurons innervating the skin and viscera, and in response to a change of ambient temperature, changes in the activity of these neurons orchestrate behavioral and autonomic responses that effectively preserve thermal homeostasis (Morrison, 2016). The afferent pathway that transduces this cutaneous thermosensory information to the brain involves neuronal cell bodies in the dorsal horn of the spinal cord that project to the lateral parabrachial nucleus (LPBN) and from there to the hypothalamic median preoptic nucleus (MnPO), located within the POA. Available data suggest that these MnPO neurons in turn, synapse onto, and thereby inhibit warm-sensitive, GABAergic neurons located elsewhere in the POA, and that these inhibitory neurons project onto neurons in the DMN that control the output of downstream sympathetic premotor neurons that control SNS outflow to thermogenic tissues. During cold exposure, therefore, activation of cold-responsive MnPO neurons in response to cutaneous thermosensory input is proposed to inhibit the activity of warm-sensitive, GABAergic neurons in the POA, an effect that ultimately increases heat production by disinhibiting DMN neurons that drive sympathetic outflow (Morrison, 2016). Consistent with previous evidence that at least some of these POA neurons are themselves thermosensitive, we found that localized POA cooling raises core body temperature, while POA warming has the opposite effect, consistent with published evidence that this response is dependent on increased sympathetic outflow to BAT as is

observed during cold exposure (Mohammed et al., 2018; Nakamura and Morrison, 2007). Thus, POA cooling and cold exposure appear to engage the same set of adaptive thermogenic responses.

Our data suggest that although many adaptive responses are indeed similar, there are some differences exist where effects on glucose homeostasis are concerned. Specifically, we report that like cold exposure, intense hypothalamic POA cooling for 8 h had no effect on glucose tolerance even though glucose-stimulated insulin secretion was reduced, evidently because this effect was offset by a tendency towards increased insulin sensitivity. We note that the latter effect was blunted in comparison to what we have observed during cold exposure (Morton et al., 2017), possibly owing to the confounding effect of an associated stress response.

Published literature suggests that the cold exposure increases insulin sensitivity in thermogenic tissues such as BAT and skeletal muscle, but not in non-thermogenic tissues such as liver (Gasparetti et al., 2003; Smith et al., 1986), which allows the liver to continue to supply the glucose (via increased rates of glucose production) needed to meet the increased energy demands of thermogenic tissues whose heat production increases so as to maintain core temperature during cold exposure, while at the same time preserving normoglycemia. Several findings implicate the SNS in this effect: 1) cold exposure increases sympathetic tone to thermogenic tissues (Dulloo et al., 1988; Young et al., 1982), 2) cold-induced thermogenic responses require increased SNS outflow, 3) activation of the SNS both promotes glucose uptake into thermogenic tissues and increases hepatic glucose production (Nonogaki, 2000), and 4) the effect of cold exposure to improve insulin sensitivity is rapidly reversed by administration of the alpha-adrenergic receptor antagonist phentolamine (Morton et al., 2017). That reduced insulin secretion in this setting is also mediated by the SNS is supported by evidence that cold exposure increases sympathetic outflow to the islet (Young and Landsberg, 1979) and that the effect of cold exposure to inhibit glucose-stimulated insulin secretion is also rapidly reversed by phentolamine (Morton et al., 2017).

Because of the likelihood that metabolic adaptations to cold exposure evolve over time, we also investigated the effects of a POA cooling paradigm that was sustained over a longer time interval (over 24 h period). Unlike the effects of both cold exposure and short-term, intense POA cooling, this intervention robustly improved glucose tolerance, presumably resulting from increased AIR_G , combined with a tendency for both insulin sensitivity, such that the overall effect of insulin on glucose disposal (*i.e.*, the Disposition Index) increased by >2-fold. In addition, insulin-independent glucose disposal (*i.e.*, glucose effectiveness) also tended to increase. By comparison, we have previously shown that although cold exposure also increases insulin sensitivity, it does not improve glucose tolerance because the effect is offset by reduced insulin secretion such that the Disposition Index remains the same (Morton et al., 2017). Surprisingly this intervention also failed to decrease basal levels of either blood glucose or plasma insulin, effects observed during cold exposure (Morton et al., 2017). Combined with evidence that thermode-induced cooling of the POA (Kimura et al., 2001) induces c-fos expression (a marker of neuron activation) in brain areas different from those activated during cold exposure (Bratincsak and Palkovits, 2004), we conclude that

local POA cooling engages glucoregulatory responses both similar to and distinct from those that characterize the physiological response to cold exposure.

One potential explanation for these differences is that core body temperature does not ordinarily change during cold exposure; indeed, the goal of adaptive responses in this setting are to preserve stability of the core temperature, and under usual circumstances, this is what is observed. Thus, the adaptive thermogenic response to cold exposure cannot ordinarily be attributed to changes of local POA temperature, since core body temperature typically does not change unless cold exposure is sufficiently prolonged or severe to overwhelm the thermoregulatory system and thereby lower core temperature sufficiently to activate thermosensitive neurons in the POA. In contrast, POA cooling raises core body temperature, presumably, by “misleading” the thermoregulatory system that the body is cooler than it actually is. Stated differently, POA cooling likely activates responses resembling those engaged by cold exposure that is sufficiently prolonged or severe to overwhelm the physiological thermoregulatory system. This distinction (i.e. hyperthermia induced by POA cooling) may help to explain the associated stress response to POA cooling that in turn likely contributed to differences in the metabolic response when compared to cold exposure.

Among the thermoregulatory neuronal populations within the POA that are activated during warm exposure are those that co-express the neuropeptides PACAP and BDNF (Tan et al., 2016), and pharmacogenetic activation of these neurons mimics the effect of POA warming to induce hypothermia by engaging both autonomic (reduced BAT thermogenesis, increased tail vasodilation) and behavioral (cold seeking, reduced nest building, increased postural extension) responses. It therefore seems likely that the effect of POA cooling to increase core temperature involves inhibition of these WSNs, although the associated increase of core body temperature may offset the overall impact on whole-body glucose homeostasis. Stated differently, activation of thermoregulatory neurocircuits during POA cooling may have both direct and indirect effects on determinants glucose tolerance, effects that may partially offset one another.

Members of the transient receptor potential cation channel, TRPV1 and TRPM2 have been implicated as temperature sensors in the brain. While TRPV1 is expressed at low levels in brain and is not present in the POA (Cavanaugh et al., 2011), TRPM2 is concentrated in a subset of POA neurons that is activated at warm temperatures, and this activation plays a physiological role to limit the degree of hyperthermia associated with the fever response. Accordingly, chemogenetic activation and inhibition of hypothalamic TRPM2-expressing neurons decrease and increase core body temperature, respectively (Song et al., 2016). Interestingly, activation of these neurons by hyperthermia is hypothesized to engage downstream glutamatergic POA neurons that project onto and activate corticotropin-releasing hormone-positive (*Crh+*) neurons in the hypothalamic paraventricular nucleus that are implicated in the stress response (including hypothalamic-pituitary-adrenal (HPA) axis activation) associated with fever (Matsuoka et al., 2003; Song et al., 2016). Our finding that POA cooling markedly elevated corticosterone levels suggests that HPA axis activation may have resulted from hyperthermia (via activation of TRPM2-expressing POA neurons), thereby contributing to the observed slight increase of basal blood glucose levels and blunted increase of insulin sensitivity.

In addition, thyrotropin releasing hormone (TRH) neurons in the hypothalamic paraventricular nucleus (PVN) are implicated in physiological responses to cold exposure via activation of the hypothalamic pituitary thyroid (HPT) axis (Nillni, 2010). Cold exposure activates TRH neurons in the PVN (Cabral et al., 2012), which stimulates the secretion of thyroid-stimulating hormone (TSH) from the anterior pituitary. Increased TSH levels in turn induce thyroid hormone secretion, thereby increasing thermogenesis and stimulating glucose metabolism (Klieverik et al., 2009; Silva, 2006). In support of this concept, intracerebroventricular administration of TRH induces both hyperthermia and transient hyperglycemia (Marubashi et al., 1988), whereas intracerebroventricular administration of a TRH antibody induces hypothermia (Prasad et al., 1980). Moreover, TRH-deficient mice exhibit cold intolerance and impaired glucose metabolism (Yamada et al., 1997), and TRH administration directly into the PVN increases thermogenesis, plasma corticosterone and blood glucose levels (Zhang et al., 2018). Lastly, POA cooling increases TSH release in conscious rats (Martelli et al., 2014). Additional studies to test the role of the HPT axis in the response to POA cooling are therefore warranted.

In closing, we note that our findings have interesting, if indirect, implications for the future treatment of obesity, type 2 diabetes and related metabolic disorders. Specifically, our findings raise the possibility that if underlying neurocircuits can be targeted pharmacologically in ways that achieve beneficial glucoregulatory effects without altering core body temperature or activating the HPA axis, this type of approach may one day be employed to confer metabolic benefit to affected individuals. This notion is consistent with evidence that intermittent bouts of cold exposure 1) improve glucose homeostasis (without changes in body weight) in HFD-fed mice (Ravussin et al., 2014), 2) enhance BAT activity and cold-induced thermogenesis in humans (Yoneshiro et al., 2013), 3) increase both resting energy expenditure and whole body glucose disposal (Chondronikola et al., 2014), and 4) increase insulin sensitivity (Hanssen et al., 2015). Combined with recent evidence that like cold exposure, local POA cooling robustly increases BAT sympathetic outflow and induces BAT thermogenesis (Mohammed et al., 2018), our data support the need for studies to determine whether activation of relevant POA neurocircuits can improve glucose tolerance and insulin resistance in models of obesity, T2D or metabolic syndrome.

4. EXPERIMENTAL PROCEDURE

All animal procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University of Washington.

4.1. Animals

Adult male Wistar rats (325–350 g) were purchased from Envigo (Indianapolis, IN), individually housed in a temperature- and humidity-controlled room with a 14:10 h light/dark cycle and given ad libitum access to a standard rodent chow diet (PMI Nutrition, St. Louis, MO) and water, unless otherwise stated.

4.2. Surgery

Rats underwent implantation of a thermode directed to the hypothalamic POA under isoflurane anesthesia. The thermode was constructed from a commercially available guide cannula (20G, 10 mm; PlasticsOne, VA) that was encased with polyvinyl chloride (PVC) tubing for insulation except at the tip, which was sealed with thermally conductive silver solder. Inlet (25G, 25 mm) and outlet (20G, 9 mm) stainless steel tubing was inserted into the cannula cap unit and fixed with waterproof epoxy adhesive (Fig. 1A). Thermode patency was assessed by perfusion of room temperature water prior to the implantation surgeries. To target the POA, the thermode was implanted at stereotaxic co-ordinates: 0.0 mm from bregma, 0.5 mm lateral to midline, and 9.0 mm below the skull surface and was anchored to the skull with bridged anchor screws and acrylic cement. A thermocouple was also placed at the tip of the hypothalamic thermode to measure local hypothalamic temperature. To accomplish this, a guide cannula (26G, 12 mm; PlasticsOne, VA) was inserted into the ventral pallidum area (0.0 mm from bregma, 4.0 mm lateral to midline, and 7.7 mm below the skull surface at 15° angle) (Fig. 1B). The tip of the thermocouple was designed to extend 1.5 mm from the end of the guide cannula and correct placement was verified by injecting Chicago Sky Blue 6B dye (Millipore Sigma, MO) at study completion.

For studies involving frequently sampled glucose tolerance tests (FSIGTs), rats underwent surgical implantation of indwelling catheters (Instech Laboratories, Inc., PA) into the jugular vein and carotid artery as previously described (German et al., 2011; Morton et al., 2017; Rojas et al., 2015). Animals were given at least a 1-wk post-surgical recovery period prior to thermode and thermocouple implantation, followed by another 1-wk recovery before studies were commenced. For all surgical procedures, animals received a peri-operative subcutaneous injection of buprenorphine hydrochloride (0.05 g/g of body weight) (Reckitt Benckiser Pharmaceuticals Inc., VA).

To determine the effect of POA cooling on body temperature in conscious rats, continuous monitoring of core body temperature (every 10 minutes) was performed before (10 min) during (60 min) and after (60 min) POA cooling or warming in separate cohorts of rats at least 1 wk after intraperitoneal implantation of temperature transponder (Starr Life Science Corp, PA). Signals emitted by body temperature transponders were sensed by a receiver positioned beneath the cage and analyzed using VitalView software, as previously described (Kaiyala et al., 2015; Kaiyala et al., 2016), with support of the NIDDK-funded Nutrition Obesity Research Center Energy Balance Core at the University of Washington.

4.3. Hypothalamic POA cooling and warming

The POA was cooled or warmed by using a peristaltic pump to perfuse water through coiled plastic tubing (~20 m) that was submerged in either ice-cold, heated or room temperature water prior to entry into the thermode. As expected, pilot studies showed that hypothalamic temperature was reduced in a manner that was dependent on both the water temperature and flow rate (owing to the high thermal capacity of water). Based on this information, flow rates of either 1 or 4 mL/min were selected to robustly and reliably reduce POA temperature.

For short-term, intense POA cooling studies, animals fasted for 16 h were subjected to POA cooling by perfusing the thermode with ice-cold water ($\sim 4^{\circ}\text{C}$) at a flow rate of 4 mL/min for up to 8 h. For longer-term, moderate POA cooling, the thermode was perfused with room-temperature water ($\sim 22^{\circ}\text{C}$) at a rate of 1 mL/min for 24h. For both protocols, controls underwent thermode implantation, but the thermode was not perfused in order to ensure that POA temperature did not change.

4.4. Frequently sampled intravenous glucose tolerance test (FSIGT)

The FSIGT was performed in fasted rats following and during POA cooling described above using an established protocol (Morton et al., 2017; Rojas et al., 2015) in which serial blood sampling over time is performed via a surgically implanted carotid arterial catheter in unrestrained, conscious animals. Briefly, at $t=0$ min, rats received a glucose bolus (50% dextrose, 1 g/kg body weight) via an indwelling jugular venous catheter. Arterial blood samples (40 μL) were collected at -10, 0, 2, 5, 8, 12, 20, 30, 60 min for plasma hormone analysis and measurement of blood glucose using a hand-held glucometer. Additional samples were obtained for measurement of blood glucose levels at 1, 3, 4, 6, 10, 14, 16, 18, 25, 40 and 50 min via an arterial line. Glucose and insulin area under the curves (AUCs) were calculated based on incremental area above the baseline ($t=0$) using the trapezoid rule. To compare the contribution of 1st phase insulin secretion to glucose tolerance during the period of FSIGTs with 2nd phase insulin secretion, insulin AUC data were obtained from a separate analysis of early (0–12 or 0–20 min) and late (12–60 or 20–60 min) data sets.

4.5. Minimal Model Analysis

Minimal model analysis of blood glucose and plasma insulin levels generated from FSIGTs was carried out using the MinMod software to quantify insulin sensitivity (S_I) and glucose effectiveness (S_G) as previously described (Alonso et al., 2012; Morton et al., 2013; Morton et al., 2017; Rojas et al., 2015). The acute insulin response to glucose (AIR_G) was calculated based on insulin values between $t = 0$ –4 min and the disposition index (DI) was calculated as the product of AIR_G and S_I .

4.6. Biochemical analysis of plasma

Arterial blood samples were collected in EDTA-coated tubes and centrifuged at $7,000 \times g$ at 4°C for 4 min to separate plasma fraction. Plasma concentrations of insulin (Crystal Chem, IL) and corticosterone (ALPCO, NH) were measured by ELISA.

4.7. Statistical analysis

Statistical analysis was performed using Prism (version 7; Graph Pad Software, CA) and the data are presented as the means $\pm$ SEM. Time-course experiments were analyzed by two-way ANOVA with Tukey's post hoc test. Student's t-test was used to compare the means in two groups. Probability values less than 0.05 was considered statistically significant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors acknowledge the technical assistance provided by Trista J. Harvey, Jarrell T. Nelson, Loan Nguyen, Kayoko Ogimoto and Kevin R. Velasco at the University of Washington.

Funding: This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases Grants DK089056 (G.J.M.), DK083042, and DK101997 (M.W.S.), the NIDDK-funded Nutrition Obesity Research Center (DK035816) and the Nutrition, Obesity and Atherosclerosis (T32 HL007028) and Diabetes, Obesity and Metabolism (T32 DK007247) Training Grants at the University of Washington.

Abbreviation

SNS	sympathetic nervous system
POA	preoptic area
GSIS	glucose-stimulated insulin secretion
DI	Disposition Index
T2D	type 2 diabetes
BAT	brown adipose tissue
WSN	warm-sensitive neurons
DMN	dorsomedial nucleus
ns	not significant
FSIGT	frequently sampled intravenous glucose tolerance test
AUC	area under the curve
AIR_G	acute insulin response to glucose
S_I	insulin sensitivity index
S_G	glucose effectiveness
LPBN	lateral parabrachial nucleus
MnPO	hypothalamic median preoptic nucleus
PACAP	pituitary adenylate cyclase-activating polypeptide
BDNF	brain-derived neurotrophic factor
TRPV1	transient receptor potential cation channel subfamily V member 1
TRPM2	transient receptor potential cation channel subfamily M member 2
Crh+	corticotropin-releasing hormone-positive
HPA	hypothalamic-pituitary-adrenal
TRH	thyrotropin releasing hormone

PVN	paraventricular nucleus
HPT	hypothalamic pituitary thyroid
TSH	thyroid-stimulating hormone
HFD	high fat diet
PVC	polyvinyl chloride
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay

References

- Alonso LC, Watanabe Y, Stefanovski D, Lee EJ, Singamsetty S, Romano LC, Zou B, Garcia-Ocaña A, Bergman RN, O'Donnell CP, 2012 Simultaneous measurement of insulin sensitivity, insulin secretion, and the disposition index in conscious unhandled mice. *Obesity (Silver Spring)*. 20, 1403–12. [PubMed: 22331130]
- Banet M, Séguin JJ, 1967 A thermode for rat brain. *Electroencephalogr Clin Neurophysiol*. 23, 572–3. [PubMed: 4169830]
- Bergman RN, Ider YZ, Bowden CR, Cobelli C, 1979 Quantitative estimation of insulin sensitivity. *Am J Physiol*. 236, E667–77. [PubMed: 443421]
- Bergman RN, Phillips LS, Cobelli C, 1981 Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. *J Clin Invest*. 68, 1456–67. [PubMed: 7033284]
- Bratincsák A, Palkovits M, 2004 Activation of brain areas in rat following warm and cold ambient exposure. *Neuroscience*. 127, 385–97. [PubMed: 15262329]
- Buchanan TA, Metzger BE, Freinkel N, Bergman RN, 1990 Insulin sensitivity and B-cell responsiveness to glucose during late pregnancy in lean and moderately obese women with normal glucose tolerance or mild gestational diabetes. *Am J Obstet Gynecol*. 162, 1008–14. [PubMed: 2183610]
- Bukowiecki LJ, 1989 Energy balance and diabetes. The effects of cold exposure, exercise training, and diet composition on glucose tolerance and glucose metabolism in rat peripheral tissues. *Can J Physiol Pharmacol*. 67, 382–93. [PubMed: 2667731]
- Cabral A, Valdivia S, Reynaldo M, Cyr NE, Nilni EA, Perello M, 2012 Short-term cold exposure activates TRH neurons exclusively in the hypothalamic paraventricular nucleus and raphe pallidus. *Neurosci Lett*. 518, 86–91. [PubMed: 22580206]
- Carlisle HJ, 1966 Behavioural significance of hypothalamic temperature-sensitive cells. *Nature*. 209, 1324–5. [PubMed: 5956045]
- Cavanaugh DJ, Chesler AT, Jackson AC, Sigal YM, Yamanaka H, Grant R, O'Donnell D, Nicoll RA, Shah NM, Julius D, Basbaum AI, 2011 Trpv1 reporter mice reveal highly restricted brain distribution and functional expression in arteriolar smooth muscle cells. *J Neurosci*. 31, 5067–77. [PubMed: 21451044]
- Chondronikola M, Volpi E, Børsheim E, Porter C, Annamalai P, Enerbäck S, Lidell ME, Saraf MK, Labbe SM, Hurren NM, Yfanti C, Chao T, Andersen CR, Cesani F, Hawkins H, Sidossis LS, 2014 Brown adipose tissue improves whole-body glucose homeostasis and insulin sensitivity in humans. *Diabetes*. 63, 4089–99. [PubMed: 25056438]
- Dulloo AG, Young JB, Landsberg L, 1988 Sympathetic nervous system responses to cold exposure and diet in rat skeletal muscle. *Am J Physiol*. 255, E180–8. [PubMed: 3407770]
- Gale CC, Mathews M, Young J, 1970 Behavioral thermoregulatory responses to hypothalamic cooling and warming in baboons. *Physiol Behav*. 5, 1–6. [PubMed: 5538394]

- Gasparetti AL, de Souza CT, Pereira-da-Silva M, Oliveira RL, Saad MJ, Carneiro EM, Velloso LA, 2003 Cold exposure induces tissue-specific modulation of the insulin-signalling pathway in *Rattus norvegicus*. *J Physiol*. 552, 149–62. [PubMed: 12897167]
- German JP, Thaler JP, Wisse BE, Oh-I S, Sarruf DA, Matsen ME, Fischer JD, Taborsky GJ, Schwartz MW, Morton GJ, 2011 Leptin activates a novel CNS mechanism for insulin-independent normalization of severe diabetic hyperglycemia. *Endocrinology*. 152, 394–404. [PubMed: 21159853]
- Hammel HT, Hardy JD, Fusco MM, 1960 Thermoregulatory responses to hypothalamic cooling in unanesthetized dogs. *Am J Physiol*. 198, 481–6. [PubMed: 14399311]
- Hanssen MJ, Hoeks J, Brans B, van der Lans AA, Schaart G, van den Driessche JJ, Jörgensen JA, Boekschoten MV, Hesselink MK, Havekes B, Kersten S, Mottaghy FM, van Marken Lichtenbelt WD, Schrauwen P, 2015 Short-term cold acclimation improves insulin sensitivity in patients with type 2 diabetes mellitus. *Nat Med*. 21, 863–5. [PubMed: 26147760]
- Imai-Matsumura K, Nakayama T, 1987 The central efferent mechanism of brown adipose tissue thermogenesis induced by preoptic cooling. *Can J Physiol Pharmacol*. 65, 1299–303. [PubMed: 3621079]
- Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP, 1993 Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. *Diabetes*. 42, 1663–72. [PubMed: 8405710]
- Kaiyala KJ, Ogimoto K, Nelson JT, Schwartz MW, Morton GJ, 2015 Leptin signaling is required for adaptive changes in food intake, but not energy expenditure, in response to different thermal conditions. *PLoS One*. 10, e0119391. [PubMed: 25756181]
- Kaiyala KJ, Ogimoto K, Nelson JT, Muta K, Morton GJ, 2016 Physiological role for leptin in the control of thermal conductance. *Mol Metab*. 5, 892–902. [PubMed: 27689002]
- Kimura H, Yoshida K, Nagashima K, Kanosue K, 2001 Fos expression induced by local warming or cooling of the preoptic area in rats. *Australian Physiological Society Proceedings*. 32, 76.
- Klieverik LP, Janssen SF, van Riel A, Foppen E, Bisschop PH, Serlie MJ, Boelen A, Ackermans MT, Sauerwein HP, Fliers E, Kalsbeek A, 2009 Thyroid hormone modulates glucose production via a sympathetic pathway from the hypothalamic paraventricular nucleus to the liver. *Proc Natl Acad Sci U S A*. 106, 5966–71. [PubMed: 19321430]
- Magoun HW, Harrison F, Brobeck JR, Ranson SW, 1938 Activation of heat loss mechanisms by local heating of the brain. Vol. 1, ed. eds., *Journal of Neurophysiology*, pp. 101–114.
- Martelli D, Luppi M, Cerri M, Tupone D, Mastrotto M, Perez E, Zamboni G, Amici R, 2014 The direct cooling of the preoptic-hypothalamic area elicits the release of thyroid stimulating hormone during wakefulness but not during REM sleep. *PLoS One*. 9, e87793. [PubMed: 24498374]
- Marubashi S, Kunii Y, Tominaga M, Sasaki H, 1988 Modulation of plasma glucose levels by thyrotropin-releasing hormone administered intracerebroventricularly in the rat. *Neuroendocrinology*. 48, 640–4. [PubMed: 2977989]
- Matsuoka Y, Furuyashiki T, Bito H, Ushikubi F, Tanaka Y, Kobayashi T, Muro S, Satoh N, Kayahara T, Higashi M, Mizoguchi A, Shichi H, Fukuda Y, Nakao K, Narumiya S, 2003 Impaired adrenocorticotrophic hormone response to bacterial endotoxin in mice deficient in prostaglandin E receptor EP1 and EP3 subtypes. *Proc Natl Acad Sci U S A*. 100, 4132–7. [PubMed: 12642666]
- Mohammed M, Madden CJ, Burchiel KJ, Morrison SF, 2018 Preoptic area cooling increases the sympathetic outflow to brown adipose tissue (BAT) and BAT thermogenesis. *Am J Physiol Regul Integr Comp Physiol*.
- Moran A, Jacobs DR, Steinberger J, Hong CP, Prineas R, Luepker R, Sinaiko AR, 1999 Insulin resistance during puberty: results from clamp studies in 357 children. *Diabetes*. 48, 2039–44. [PubMed: 10512371]
- Morrison SF, Madden CJ, Tupone D, 2014 Central neural regulation of brown adipose tissue thermogenesis and energy expenditure. *Cell Metab*. 19, 741–56. [PubMed: 24630813]
- Morrison SF, 2016 Central control of body temperature. *F1000Res*. 5.

- Morton GJ, Matsen ME, Bracy DP, Meek TH, Nguyen HT, Stefanovski D, Bergman RN, Wasserman DH, Schwartz MW, 2013 FGF19 action in the brain induces insulin-independent glucose lowering. *J Clin Invest.* 123, 4799–808. [PubMed: 24084738]
- Morton GJ, Muta K, Kaiyala KJ, Rojas JM, Scarlett JM, Matsen ME, Nelson JT, Acharya NK, Piccinini F, Stefanovski D, Bergman RN, Taborsky GJ, Kahn SE, Schwartz MW, 2017 Evidence That the Sympathetic Nervous System Elicits Rapid, Coordinated, and Reciprocal Adjustments of Insulin Secretion and Insulin Sensitivity During Cold Exposure. *Diabetes.* 66, 823–834. [PubMed: 28115396]
- Nakamura K, Morrison SF, 2007 Central efferent pathways mediating skin cooling-evoked sympathetic thermogenesis in brown adipose tissue. *Am J Physiol Regul Integr Comp Physiol.* 292, R127–36. [PubMed: 16931649]
- Nakamura K, Morrison SF, 2011 Central efferent pathways for cold-defensive and febrile shivering. *J Physiol.* 589, 3641–58. [PubMed: 21610139]
- Nillni EA, 2010 Regulation of the hypothalamic thyrotropin releasing hormone (TRH) neuron by neuronal and peripheral inputs. *Front Neuroendocrinol.* 31, 134–56. [PubMed: 20074584]
- Nonogaki K, 2000 New insights into sympathetic regulation of glucose and fat metabolism. *Diabetologia.* 43, 533–49. [PubMed: 10855527]
- Prasad C, Jacobs JJ, Wilber JF, 1980 Immunological blockade of endogenous thyrotropin-releasing hormone produces hypothermia in rats. *Brain Res.* 193, 580–3. [PubMed: 6770963]
- Ravussin Y, Xiao C, Gavrilova O, Reitman ML, 2014 Effect of intermittent cold exposure on brown fat activation, obesity, and energy homeostasis in mice. *PLoS One.* 9, e85876. [PubMed: 24465761]
- Rojas JM, Matsen ME, Mundinger TO, Morton GJ, Stefanovski D, Bergman RN, Kaiyala KJ, Taborsky GJ, Schwartz MW, 2015 Glucose intolerance induced by blockade of central FGF receptors is linked to an acute stress response. *Mol Metab.* 4, 561–8. [PubMed: 26266088]
- Silva JE, 2006 Thermogenic mechanisms and their hormonal regulation. *Physiol Rev.* 86, 435–64. [PubMed: 16601266]
- Smith SA, Young P, Cawthorne MA, 1986 Quantification in vivo of the effects of insulin on glucose utilization in individual tissues of warm- and cold-acclimated rats. *Biochem J.* 237, 789–95. [PubMed: 3541903]
- Song K, Wang H, Kamm GB, Pohle J, Reis FC, Heppenstall P, Wende H, Siemens J, 2016 The TRPM2 channel is a hypothalamic heat sensor that limits fever and can drive hypothermia. *Science.* 353, 1393–1398. [PubMed: 27562954]
- Stefanovski D, Richey JM, Woolcott O, Lottati M, Zheng D, Harrison LN, Ionut V, Kim SP, Hsu I, Bergman RN, 2011 Consistency of the disposition index in the face of diet induced insulin resistance: potential role of FFA. *PLoS One.* 6, e18134. [PubMed: 21479217]
- Tan CL, Cooke EK, Leib DE, Lin YC, Daly GE, Zimmerman CA, Knight ZA, 2016 Warm-Sensitive Neurons that Control Body Temperature. *Cell.* 167, 47–59.e15. [PubMed: 27616062]
- Vallerand AL, Lupien J, Bukowiecki LJ, 1983 Interactions of cold exposure and starvation on glucose tolerance and insulin response. *Am J Physiol.* 245, E575–81. [PubMed: 6362428]
- Yamada M, Saga Y, Shibusawa N, Hirato J, Murakami M, Iwasaki T, Hashimoto K, Satoh T, Wakabayashi K, Taketo MM, Mori M, 1997 Tertiary hypothyroidism and hyperglycemia in mice with targeted disruption of the thyrotropin-releasing hormone gene. *Proc Natl Acad Sci U S A.* 94, 10862–7. [PubMed: 9380725]
- Yoneshiro T, Aita S, Matsushita M, Kayahara T, Kameya T, Kawai Y, Iwanaga T, Saito M, 2013 Recruited brown adipose tissue as an antiobesity agent in humans. *J Clin Invest.* 123, 3404–8. [PubMed: 23867622]
- Young JB, Landsberg L, 1979 Effect of diet and cold exposure on norepinephrine turnover in pancreas and liver. *Am J Physiol.* 236, E524–33. [PubMed: 443373]
- Young JB, Saville E, Rothwell NJ, Stock MJ, Landsberg L, 1982 Effect of diet and cold exposure on norepinephrine turnover in brown adipose tissue of the rat. *J Clin Invest.* 69, 1061–71. [PubMed: 7068845]
- Zhang Z, Machado F, Zhao L, Heinen CA, Foppen E, Ackermans MT, Zhou J, Bisschop PH, Boelen A, Fliers E, Kalsbeek A, 2018 Administration of Thyrotropin-Releasing Hormone in the

Hypothalamic Paraventricular Nucleus of Male Rats Mimics the Metabolic Cold Defense Response. *Neuroendocrinology*. 107, 267–279. [PubMed: 30092582]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Highlights

- Hypothalamic preoptic area (POA) cooling induces thermogenic responses typically observed during cold exposure in rats.
- Short-term POA cooling mimics aspects of the glucoregulatory response to cold exposure.
- Long-term POA cooling improves glucose tolerance.

Figure 1. Validation of the water-perfused thermode system for POA cooling.

(A) Picture (left) and diagram (right) of the thermode. (B) Placement of a thermode into the POA and location of the tip of a thermocouple. (C) Changes of hypothalamic temperature during either POA moderate cooling, POA intense cooling or POA warming that was induced by the perfusion of room temperature, ice-cold or heated water into a thermode in anesthetized rats, respectively (n=4–6). * p<0.05 vs. t=0. (D) Thermogenic responses to hypothalamic POA moderate cooling, intense cooling or warming in conscious rats (n=4–5) relative to either no perfusion control or no thermode. * p<0.05 vs. No thermode and No

perfusion. (E) 7-day weight gain (n=7) and (F) 7-day food intake (n=7) in rats with or without a thermode.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Figure 2. Time-course changes of blood glucose and plasma insulin during cold exposure (5°C) relative to room temperature conditions.

Changes in (A) blood glucose and (B) plasma insulin levels over a 28 h period (n=4). * p<0.05 vs. Room temp.

Figure 3. Effect of 8h intense POA cooling on metabolic parameters.

(A) Schematic of study design examining the effect of intense POA cooling on determinants of glucose tolerance using a FSIGT. Blood glucose (B), plasma insulin (C) and plasma corticosterone levels (D) at baseline and following 8h intense POA cooling relative to no perfusion control (n=8–9). * p<0.05 vs. No perfusion.

Figure 4. Effects of intense POA cooling for 8h on determinants of glucose tolerance.

(A) Blood glucose levels, (B) the change in blood glucose levels, (C) glucose area under the curve (AUC), (D) plasma insulin levels, (E) the change in plasma insulin levels, (F) inverse area under the curve and determination of the (G) acute insulin response to glucose (AIR_G), (H) insulin Sensitivity Index (S_I), (I) Disposition Index (DI) and (J) Glucose Effectiveness (S_G) using Minimal Model Analysis in fasted male Wistar rats that were exposed to 8h intense POA cooling relative to no perfusion control and subjected to a FSIGT (n=8–9). * p<0.05 vs. No perfusion.

Figure 5. Effect of 24 h moderate POA cooling on metabolic parameters.
 (A) Schematic of study design examining the effect of moderate POA cooling on determinants of glucose tolerance using a FSIGT. Blood glucose (B), plasma insulin (C) and plasma corticosterone levels (D) at baseline and following 24h moderate POA cooling relative to no perfusion control (n=8–9). * p<0.05 vs. No perfusion.

Figure 6. Effects of moderate POA cooling for 24h on determinants of glucose tolerance during a FSIGT.

(A) Blood glucose levels, (B) the change in blood glucose levels, (C) glucose area under the curve (AUC), (D) plasma insulin levels, (E) the change in plasma insulin levels, (F) inverse area under the curve and determination of the (G) acute insulin response to glucose (AIR_G), (H) insulin Sensitivity Index (S_I), (I) Disposition Index (DI) and (J) Glucose Effectiveness (S_G) using Minimal Model Analysis in fasted male Wistar rats that were exposed to 24h moderate POA cooling relative to no perfusion control and subjected to a FSIGT (n=5–6). * $p < 0.05$ vs. No perfusion.