Profiling Postprandial Thermogenesis in Muscle and Fat of Sheep and the Central Effect of Leptin Administration

Belinda A. Henry, Frank R. Dunshea, Merryn Gould, and Iain J. Clarke

Department of Physiology (B.A.H., M.G., I.J.C.), Monash University, Victoria 3800, Australia; and Faculty of Land and Food Resources (F.R.D.), The University of Melbourne, Parkville, Victoria 3010, Australia

Brown adipose tissue thermogenesis is an important component of energy expenditure as exemplified in rodents. Other tissues such as white adipose tissue and muscle are also capable of thermogenesis, but regulation of heat production in these tissues is poorly understood. We used a relatively large animal model, the ovariectomized sheep, in which site-specific temperature measurements were made as an index of thermogenic output. Dataloggers were implanted into the retroperitoneal (visceral) fat, gluteal (sc) fat, and skeletal muscle of the hind limb, and were programmed to record temperature every 15 min. Animals (n = 4) were then placed on a feeding schedule (fed between 1100 and 1600 h) to entrain a postprandial response in thermogenesis. Baseline thermogenesis (0800–1100 h) was higher (P < 0.05) in visceral fat and muscle than in gluteal fat, whereas the amplitude of the postprandial response was similar at all three sites. Intracerebroventricular infusion into the lateral ventricle of either vehicle (artificial cerebrospinal fluid) or leptin (10 μg/h at 100 μl/h) for 24 h (0900–0900) was performed in a cross-over design with a 1-wk recovery period between treatments. Central leptin infusion did not alter the basal thermogenic rate but markedly enhanced the postprandial response in both fat and muscle tissues. This was manifest by increased (P < 0.05) amplitude and duration of the postprandial thermogenic response, and the effect was greater in muscle and visceral fat than in gluteal fat. These data demonstrate that leptin is able to regulate thermogenesis in muscle, providing a novel target for the manipulation of energy balance. (Endocrinology 149: 2019–2026, 2008)

THE RATE AT which energy is expended is integral to the determination of body weight and the degree of adiposity of animals. There are two broad categories of energy expenditure: that involved in the maintenance of basic cellular function and metabolism, and that related to physical activity and adaptive thermogenesis. The brain regulates adaptive thermogenesis in response to cold or dietary stimuli (1). The thermic effect of food, a component of which comprises thermogenesis, is a well-characterized phenomenon in humans and animals (1, 2). Diet-induced thermogenesis accounts for around 15% of energy expenditure in a nonobese individual, although this response to feeding is blunted in obese individuals (3).

In rodents, diet-induced thermogenesis occurs in brown adipose tissue (BAT), in response to activation of the sympathetic nervous system. The release of noradrenaline at sympathetic neuronal terminals on BAT adipocytes activates uncoupling protein (UCP) 1, which extricates protons from the oxidative cycle resulting in the dissipation of energy through the production of heat. There has been significant focus on thermogenesis in BAT as a component of whole animal energy expenditure and regulation of body weight, but it is not clear how data from rodents translate to humans. In humans, diet-induced or postprandial thermogenesis is an important component of the thermic effect of food (4). Furthermore, BAT exists in adult humans, with brown adipocytes being interspersed among white adipocytes (5–7). What remains to be elucidated, is to what extent these diffuse fat beds contribute to energy expenditure via thermogenic functions in nonrodent species.

Another issue that is unresolved in terms of thermogenesis is the extent to which tissues other than fat may contribute. In humans, one can account for inherent individual variation in basal metabolic rate through differences in resting energy expenditure of skeletal muscle (8). Indeed, studies in humans have demonstrated that skeletal muscle is largely responsible for the adrenaline-induced changes in thermogenesis; skeletal muscle accounts for 40–50% of the increase in thermogenesis, whereas changes attributable to fat are around 5% or less (9, 10). Because skeletal muscle represents 30–40% of total body mass, changes in energy expended by this tissue are likely to have a major impact on whole body energy homeostasis, eclipsing the contribution of tissues such as adipose. Despite this, very little is known about factors that exert central actions to regulate thermogenesis in muscle.

In rodents, the adipocyte-derived hormone, leptin regulates thermogenesis in BAT directly and indirectly by controlling sympathetic outflow from the brain (1). In rats, catabolic states such as starvation (11) and lactation (12) reduce UCP1 mRNA expression in BAT, and this effect can be reversed by peripheral leptin treatment. Central administration of leptin (either intracerebroventricular or by injection into the arcuate nucleus of the hypothalamus) to anesthetized rats increases sympathetic nerve activity in BAT (13). Central administration of a leptin antagonist to rats blunted high-fat feeding-induced elevation of UCP1
mRNA expression in BAT (14), providing compelling evidence that leptin acts on the brain to regulate diet-induced thermogenesis. In contrast, effects of leptin on skeletal muscle thermogenesis remain ambiguous. Using ex vivo explants of murine soleus muscle, leptin treatment increased oxygen uptake (15), indicative of thermogenesis and providing evidence for a direct effect on muscle in this species. Regulation of muscle thermogenesis by the brain has not been described. Accordingly, we aimed to determine the effect of central leptin administration on the postprandial increase in temperature in discrete adipose (visceral fat and sc fat) and skeletal muscle.

The adult sheep represents a model of thermogenesis similar to that of the adult human, whereby brown adipocytes are interspersed among white adipocytes, as demonstrated by the presence of UCP1 mRNA (16). Furthermore, due to the relative size of the sheep, temperature probes can be used to profile thermogenesis in discrete tissue depots with ease. Here, we describe the establishment of a model of diet-induced entrainment of a thermogenic response in adipose tissue and in muscle. This closely resembles the general pattern of food intake in humans, which is at “programmed” meal times rather than being continuous. We determined the effect of leptin action on the brain to manipulate postprandial thermogenesis in skeletal muscle, as well as visceral and sc fat.

Materials and Methods

Animals

All experimentation was approved by the relevant ethics committee of Monash University. Four ewes (58.1 ± 2.7 kg) were ovariectomized approximately 6 months before experimentation. Customized dataloggers with either 10 or 20-cm leads (SubCue, Calgary, Canada) were implanted into three sites for temperature measurements: the retroperitoneal fat bed (20-cm lead), sc gluteal fat (10-cm lead), and into the muscle of the hind limb (10-cm lead). For placement in muscle, the vastus lateralis and the biceps femoris muscles were separated by blunt dissection, and the datalogger was placed with the recording side facing biceps femoris. For placement in the retroperitoneal fat, care was taken to place the datalogger in fat that was not in close proximity to the kidney so that recordings were not influenced by kidney function or blood flow. In each case, the datalogger was anchored into position using silk suture, the lead and download point were exteriorized, and the wound sutured. Animals were then placed on a temporal feeding regime to entrain a postprandial thermogenic response in adipose tissue and in muscle. This closely represents the general pattern of food intake in humans, which is at “programmed” meal times rather than being continuous. We determined the effect of leptin action on the brain to manipulate postprandial thermogenesis in skeletal muscle, as well as visceral and sc fat.

To demonstrate the specificity of the postprandial response, a group of ovariectomized ewes (n = 5) received dataloggers in the retroperitoneal fat, and the temperature profiles were followed. Once the postprandial response was entrained (after 1-wk feeding at 1200 h), the animals were fasted for 24 h, to ascertain whether there was a “feeding” response at the scheduled meal (1200 h). This experiment was such that any component of temperature excursion that was related to diurnal programming would have been evident, in the absence of feeding.

Lateral ventricular infusion of leptin

Lateral ventricular cannulation was performed at least 1 month before the beginning of experimentation. For central infusion, SILASTIC brand cannulae (Dow Corning, Corp., Midland, MI; inner diameter = 0.64 mm, outer diameter = 1.19 mm, length = 250 mm) were inserted into the lateral ventricle. In brief, the skin is opened over and behind bregma, extending caudally over a distance of 30 mm. A burr hole was made 5-mm lateral to the midline at the level of the parietal/occipital fissure, and the infusion line was introduced through the dura mater to a depth of 15 mm. Access to the lateral ventricle was facilitated by the flow of cerebrospinal fluid (CSF). Placement of the cannula, above the foramen of Munro, was confirmed by injection of radioopaque dye (1 ml) and a lateral ventriculogram x-ray image. When in place, the infusion line was secured with Super Glue (Super Glue Corp., Rancho Cucamonga, CA), the line capped, and tunneled under the skin approximately 10 cm. For access, 5 cm of the cannulae was exteriorized behind the neck.

Recombinant human leptin was synthesized and purified as described previously (17). For infusion, leptin was dissolved in 1 mM HCl (0.5 mg/ml) and then diluted in vehicle (artificial CSF: 150 mM NaCl, 1.2 mM CaCl2, 1 mM MgCl2, and 2.8 mM KCl). Vehicle and leptin were administered using Graseby M16A microinfusion pumps (Graseby Medical Ltd., Gold Coast, Australia), and 3-ml syringes were connected to the ventricular cannulae. Either leptin (10 μg/h) or artificial CSF infusion was at a rate of 100 μl/h for 24 h beginning at 0900 h. We used a cross-over design with 1 wk between reverse treatments (n = 4 for each treatment).

![Fig. 1. Innate differences in thermogenic rates in different tissues in the sheep. Baseline thermogenesis (0800–1100 h) is lower in the gluteal fat compared with either the visceral (retroperitoneal) fat or skeletal muscle (upper panel). All three sites were responsive to a meal that entrained postprandial thermogenesis (lower panel), which was similar in amplitude (Amp). * Compared with gluteal fat (P <0.05). Temp, temperature.](https://academic.oup.com/endo/article-lookup/149/4/2019/2455370)
On the day before infusion, one external jugular vein was cannulated, extended with a manometer line (Portex Ltd., Kent, UK) and closed with a three-way tap. On the day of infusion, blood samples (6 ml) were taken and collected into heparinized tubes, centrifuged at 4 C, and stored at −20 C until assayed. Samples were taken hourly between 1000 and 1500 h, and used to characterize plasma nonesterified fatty acid (NEFA) concentration (18). Plasma glucose levels were measured in a single assay (procedure 510A; Sigma Chemicals, St. Louis, MO). Core body temperatures were taken hourly between 0900 and 1500 h, using a rectal thermometer.

**Data and statistical analyses**

Effects of leptin on thermogenic output was characterized by analyzing effects on baseline temperature (mean of 0800–1100 h) and effects of feeding (mean of 1100–1600 h). Temperatures were analyzed as the mean of 4-h increments thereafter until temperature returned to baseline values. All data were checked for homogeneity of variance, and single-factor ANOVA was used to compare baseline thermogenesis and the amplitude of the postprandial increase across the three tissue sites. Repeated measures ANOVA was used to analyze the effect of leptin treatment on the amplitude and duration of the postprandial increase in temperature, food intake, core body temperature, and plasma NEFA and glucose levels; post hoc comparison of means was made using a single-factor ANOVA (SPSS version 14.0; SPSS, Inc., Chicago, IL). Data are presented as means ± SEM.

**Results**

**Thermogenesis**

Baseline temperature (0800–1100 h) was higher (*P* < 0.05) in visceral fat and skeletal muscle than in sc gluteal fat (Fig. 1, upper panel), but the amplitude of the postprandial increase in temperature was similar in all three tissues studied (Fig. 1, lower panel).

Temperature excursions were observed in the three tissues across a 24-h period, as shown in Figs. 2 and 3. The feeding window entrained a postprandial thermogenic response, which was apparent on the day before experimentation. Thus, temperature increased by 1.5–2.5% within 8 h of the commencement of feeding. Furthermore, elevation in temperatures was specifically related to feeding (Fig. 4) because no such response was seen when the animals were fasted. Thus, no diurnal excursion in temperature was evident without feeding. Elevation of temperature was also apparent after the feeding period on the day before experimentation, but this was most likely due to removal of animals from their pens for jugular cannulation, and this did not occur on the day after experimentation.

There was no effect of artificial CSF infusion on postprandial thermogenesis in either gluteal fat (Fig. 2, upper panel), visceral fat (Fig. 2, middle panel), or skeletal muscle (Fig. 2, lower panel). Central infusion of leptin impacted on the diurnal rhythm of temperature in all three sites (Fig. 3). There was no effect of leptin on baseline temperatures, but intracerebroventricular infusion of leptin markedly increased postprandial thermogenesis (Fig. 5). Leptin increased the amplitude of the postprandial thermogenic response to a similar degree in all three sites, but the effect of leptin on the duration of the postprandial thermogenic response was site dependent. Thus, temperature levels returned to baseline values within 16 h after feeding in sc gluteal fat (Fig. 5, upper panel) and by 28 h after feeding in muscle (Fig. 5, lower panel). Visceral fat temperature after leptin treatment did not return to baseline values within the observation period (Fig. 5, middle panel).

Core body temperature was unaffected by feeding before treatment and after treatment with artificial CSF (Fig. 6). Core temperature was elevated (*P* < 0.05) in the
leptin-treated animals, but this lagged the tissue-specific elevation in presumed thermogenesis; in fat and muscle, thermogenesis increased ($P < 0.05$) at 1300 h, whereas core body temperature was increased at 1400 h. Central infusion of leptin had no effect on core body temperature during the baseline period (0900–1100 h), indicating a lack of pyrogenic effect of leptin.

Plasma NEFA levels were reduced ($P < 0.05$) at 1400 and 1500 h compared with prefeeding levels (1000 h) in control animals, but not the leptin-treated animals (Fig. 7). Plasma levels of glucose were similar in control and leptin-treated animals, however, feeding increased ($P < 0.05$) plasma glucose levels in both control and leptin-treated groups.

Intracerebroventricular infusion of leptin reduced ($P < 0.01$) food intake, and the effect persisted for the following 2 d ($P < 0.05$) (Fig. 7). Infusion of artificial CSF had no effect on food intake.

**Discussion**

We have developed a model that unmasks postprandial thermogenic responses in muscle and fat. Sheep are normally continuous feeders, but feeding within a window of time programs a thermogenic response. This provides a unique animal model in which postprandial thermogenesis is apparent, similar to the response seen in humans (4). Using this model, we show that central administration of leptin increases postprandial thermogenesis in muscle, as well as visceral and sc fat. The response in muscle is especially important because this has not been recognized up to this time and represents an important target for the manipulation of energy expenditure.

Postprandial temperature excursions were apparent in all three tissue depots examined. It is important to consider whether such responses represent thermogenesis or some component of metabolic function, such as the thermic effect of feeding. The latter is divided into two cate-
gories, with obligatory and facultative components. The obligatory component refers to energy expended during the digestion and processing of nutrients, whereas the facultative component refers to energy expended above that of the obligatory demand, i.e., thermogenesis (2). Because postprandial temperature excursions occurred in fat and muscle without an initial increase in core body temperature, this suggests that the localized effects were due to changes in thermogenic activity.

To entrain the postprandial response, animals were subjected to a regime of temporal food restriction. Previous studies in rodents have demonstrated that temporal food restriction is capable of establishing a circadian food-entrainable rhythm (19, 20). This circadian rhythm was not observed in our sheep model. In rodents, the food-entrainable oscillator is associated with an increase in core body temperature that precedes the onset of daily feeding (19, 20). The current model demonstrated that increased thermogenic activity was a true postprandial effect. An elevation in temperature in fat and muscle tissues occurred only after the onset of feeding, and was not evident in the absence of food (during a day of fasting).

Fig. 5. The effect of central leptin infusion on postprandial thermogenesis in gluteal fat (upper panel), visceral fat (middle panel), and skeletal muscle (lower panel). Animals (n = 4 per group) were treated with either leptin (open circles) or artificial CSF (aCSF) (closed circles). Intracerebroventricular infusion of leptin (10 μg/h) increased the amplitude and the duration of the postprandial response. Data are presented as means (±SEM). Time points are the average temperature across a 4-h period. a, Compared with baseline values (time -3) (P < 0.05). b, Leptin compared with artificial CSF treatments (P < 0.05).

Fig. 6. The time line between the onset of tissue-specific thermogenesis in gluteal fat, visceral fat, and skeletal muscle, as well as core body temperature. Animals (n = 4 per group) were treated with either leptin (open circles) or artificial CSF (closed circles). Central administration of leptin (10 μg/h) induced an increase in thermogenic activity in all three tissue sites at 1300 h, whereas core body temperature was not higher in leptin-treated animals until 1400 h, indicative of a true thermogenic effect. Data are presented as the means (±SEM). a, Time point compared with 0900 h in the leptin-treated animals (P < 0.05). b, Time point compared with 0900 h in the vehicle-treated animals (P < 0.05).
Inherent differences in baseline temperatures were demonstrated in gluteal fat compared with muscle and visceral fat; basal gluteal fat temperature was around 0.5°C lower ($P < 0.05$) compared with visceral fat and skeletal muscle. This suggests that sc gluteal fat exhibits innately lower thermogenic capacity than visceral fat or skeletal muscle. Despite this, the thermogenic response to feeding was similar in all three tissues; the amplitude of the postprandial increase in temperature was comparable in gluteal fat, visceral fat, and skeletal muscle. Previous studies in humans and rodents have demonstrated heterogeneity in the expression of various genes across fat depots (21–23). In neonatal sheep, brown adipocytes are most concentrated within the fat depots of the peritoneal cavity, particularly in the retroperitoneal area (24). The present data show that sc gluteal fat exhibits a lower basal thermogenic rate than retroperitoneal visceral fat, and the effect of central leptin administration on postprandial temperature excursions was lower in gluteal fat than visceral fat (see below). Although the lower temperature in gluteal fat may reflect its superficial location, this tissue exhibited decreased responsiveness to central leptin administration, indicative of innate differences in thermogenic capacity across tissue sites.

We have shown that leptin acts within the brain to elicit discrete tissue-specific enhancement of postprandial thermogenesis, which is consistent with the accepted notion of dual action to reduce food intake (17) as well as increasing energy expenditure (25). The central effect to increase the temperature of muscle and fat was not attributable to an increase in the obligatory component associated with the thermic effect of feeding because leptin actually reduced daily food intake. Importantly, leptin administration before feeding did not affect basal temperature levels in the three tissues studied, which provides support for the assertion that the effect is on postprandial thermogenesis and is not due to a pyrogenic effect of the cytokine. Moreover, effects of central leptin treatment on postprandial thermogenesis reflected the innate differences in thermogenic capacity of the three sites studied. Thus, the leptin-enhanced postprandial increase in thermogenesis persisted for 16 h after feeding in gluteal fat but was extended for longer in skeletal muscle (28 h) and visceral fat (28 h+). Therefore, skeletal muscle demonstrates an equivalent thermogenic capacity to that of visceral fat. This novel description of the effect of leptin within the brain to increase thermogenesis in skeletal muscle is most likely due to sympathetic outflow, although further studies are required to detail this mechanism. Given the proportion of skeletal muscle to body mass and its enhanced thermogenic capacity, the effect of leptin on skeletal muscle is likely to have far greater impact on daily energy expenditure and the regulation of body weight than the effect of leptin on adipose sites.

Increased thermogenesis is likely to involve UCP function, with regulation by sympathetic outflow to discrete tissues and/or regional variations in blood flow. It is well known that, in rodents, BAT is important in the process of adaptive (or facultative) thermogenesis, via modulation of UCP1 function (26). The thermogenic function of white adipose tissue is believed to be due to the presence of specialized brown adipocytes interspersed among white adipocytes (27) Both white fat and muscle express UCP2, and muscle also expresses UCP3, but the role that these play in thermogenesis is not elucidated (28). One proposition is that skeletal muscle thermogenesis is due to the presence of brown adipocytes within the fat surrounding muscle bundles, and not the muscle tissue per se (29). Despite this, various studies in rodents have clearly demonstrated that thermogenesis can occur in tissues such as white adipose and muscle in the absence of UCP1 (30–33).

Our data provide further support for the notion that thermogenesis occurs in white fat depots and in muscle, despite the comparatively low levels of UCP1 in these tissues.

It is possible that the fluctuations in temperature we observed after central leptin administration were the consequence of alterations in regional blood flow, but this appears unlikely. Previous studies in dogs showed that blood flow within skeletal muscle was not influenced by feeding, and blood flow changes associated with feeding occurred only in tissues involved in digestion (obligatory component of the thermic effect of food) (34). On the other hand, one might expect that increased temperature would lead to a physiological response in terms of blood flow, and studies are in progress to determine whether this is the case. Whether there are changes in blood flow as a consequence of changing temperature, the fact remains that temperature elevation in the tissues examined represents a dissipation of energy.
In rodents and humans, NEFA modulate the expression of UCPs (35–37). We did not observe changes in plasma NEFA or glucose levels in relation to altered thermogenesis. Before feeding (1000 h), plasma NEFA levels were highest, yet this was during the baseline period of thermogenesis. Plasma NEFA levels corresponded to the temporal feeding regime, being reduced by feeding in the control animals; this effect was prevented by leptin treatment. Feeding increased plasma glucose levels to a similar extent in both control and leptin-treated animals. The lack of effect of feeding on plasma NEFA levels in the leptin-treated animals was likely to be due to reduced food intake in this group and, therefore, the mobilization of fuel stores. Thus, in light of the reduced food intake and mobilization of NEFAs, increasing levels of plasma glucose were likely to be partially due to a gluconeogenic source in these animals. Thus, in the current study, leptin-induced thermogenesis in muscle and adipose tissue was not associated with an increase in circulating NEFAs or glucose.

In conclusion, we demonstrate that a postprandial increase in thermogenesis can be entrained in sheep by using a feeding window; this effect is evident in gluteal (sc) fat, visceral (retroperitoneal) fat, and skeletal muscle. Such a model is, to our knowledge, unique beyond the well-characterized phenomenon in humans (4). This enables a detailed study of thermogenic profiles in various body tissues in a large animal, and we have demonstrated that central infusion of leptin markedly increased postprandial thermogenesis in muscle and adipose depots. The effect is robust in skeletal muscle, which has not been considered as a major thermogenic tissue previously. Given that skeletal muscle represents a significant mass within the body, the energy expended by this tissue is of great importance in the determination of daily energy expenditure and represents a unique target for manipulation of body weight.

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Address all correspondence and requests for reprints to: Dr. Belinda Henry, Department of Physiology, Building 13 F, Wellington Road, Monash University, Victoria 3800, Australia. E-mail: belinda.henry@med.monash.edu.au.

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