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Review

Denervation as a tool for testing sympathetic control of white adipose tissue

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ABSTRACT

This review summarizes the evidence derived from studies utilizing denervation procedures to demonstrate sympathetic control of white adipose tissue metabolism and body fat mass. A majority of the work demonstrating neural control of white fat was performed in the Bartness laboratory with Siberian hamsters as the predominant experimental model. These animals experience dramatic changes in body fat mass in response to changes in photoperiod, however, the mechanisms identified in hamsters have been reproduced or further elucidated by experiments with other animal models. Evidence for the role of sympathetic innervation contributing to the control of white adipocyte lipolysis and preadipocyte proliferation is summarized. In addition, evidence from denervation experiments for neural communication between different white fat depots as well as for a feedback control loop between sensory afferents from individual fat depots and sympathetic efferents to the same or distant white fat depots is discussed.

1. Introduction

The objective of this review is to provide a summary of the work contributed by Timothy J. Bartness to the understanding of central control of adipose mass through methods that involve direct manipulation of the innervation of individual fat depots. The animal model used in a large number of these studies is the hamster (Syrian or Siberian) which can be induced to gain or lose body fat simply by changing photoperiod. Thus the change in fat is mediated by endogenous control systems that respond to a normal biological signal rather than representing a response to an unusual insult such as dietary induced weight gain or surgical loss (lipectomy) or gain (transplant) of body fat.

2. Seasonal changes in body fat of hamsters

Tim initiated his work with photoperiod in hamsters in the 1980s during his time as a postdoctoral fellow with George N. Wade at the University of Massachusetts and solidified this interest during a subsequent postdoctoral fellowship with Bruce D. Goldman at the Worcester Foundation. It was already established that photoperiod influenced adiposity in hamsters. Syrian hamsters (*Mesocricetus auratus*) gain a large amount of fat in short photoperiod, winter-like, days [1] whereas Siberian hamsters (*Phodopus sungorus*) lose fat in similar conditions [2]. The changes in body composition are fully reversible and follow the seasons when the animals are living in the wild. It is important to note that Siberian hamsters exposed to short photoperiod lose weight and then this is followed by a reduction in food intake which means that the change in body fat is a primary response rather than a secondary outcome following changes in energy balance [3]. Thus, the change in body composition is caused by a photoperiod responsive system that modulates lipolysis and/or lipogenesis.

It had already been established that the photoperiodic changes in body weight and composition were driven by the hormone melatonin released from the pineal gland during the dark period [2]. Tim completed a work intensive study with pinealectomized hamsters housed in long photoperiod and treated with melatonin and found that the changes in body weight were driven by the duration of the nocturnal peak of melatonin, rather than the actual concentration of melatonin in the circulation. Hamsters infused with 10 ng melatonin/day during a short infusion of 4 h duration gained body weight and fat comparable to that in Siberian hamsters housed in long day photoperiod. However, animals infused with the same amount of melatonin over a longer period of 10 h did not show any change in body composition [4]. In a complementary study, long duration melatonin infusions into long day housed hamsters produced responses typical of an animal housed in a short day length [5]. In male Siberian hamsters housed in short photoperiod conditions intraperitoneal fat (epididymal and retroperitoneal) decreased in size more than subcutaneous fat depots (inguinal and dorsal), but were also restored more rapidly when the animals were transferred to long photoperiod conditions [6]. This regional response was not apparent in females.

The next step in the process of understanding the seasonal control of body fat mass was to identify the melatonin-responsive signal that

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changed fat metabolism. The seasonal changes in body weight and composition of hamsters are accompanied by other physiologic adaptations including testicular regression when fat is lost and recrudescence when fat is increased [3,7,8]. An initial study tested whether the changes in fat were secondary to the change in gonadal hormone levels, but neither castration of male Siberian hamsters nor ovariectomy of females had any effect on the loss of body fat in short-day conditions [3]. This was the first in a series of studies designed to identify a hormonal mediator of the melatonin-driven photoperiodic changes in body weight, body fat and reproductive status. Subsequent depletion, repletion experiments tested prolactin [9], insulin [10], thyroxine [11] and adrenal norepinephrine as potential mediators [12]. Although none of these hormones were found to be the exclusively driver of white adipose tissue lipolysis, it did appear that the combination of sympathetic norepinephrine and adrenal epinephrine accounted for the short day loss of fat in Siberian hamsters [12]. Subsequently the Bartness lab identified melatonin receptors on sympathetic neurons originating in the suprachiasmatic nucleus and terminating in white fat depots [13]. Thus, Tim's interest in neural control of adipose tissue developed from a failure to find a hormonal explanation for the photoperiodic-dependent changes in hamsters, but the amount of work involved in the hormone studies and the frustration of obtaining only negative results remained a topic of conversation throughout his career.

The first evidence for sympathetic innervation of white fat from the Bartness lab [14] used anterograde and retrograde tract tracing to provide anatomic evidence of sympathetic and sensory innervation of different white fat depots (subcutaneous inguinal and intraperitoneal epididymal). This paper clearly showed that there was innervation of the fat cells and not just blood vessels within a depot. The specificity of the tracing was confirmed by surgical denervation of fat depots and by injecting the tracers directly into blood vessels. In this and future studies norepinephrine turnover (NETO) in individual fat pads was determined by measuring the decline in adipose norepinephrine (NE) content following inhibition of tyrosine hydroxylase with alpha-methyl-*p*-tyrosine (α-MPT). The results showed a correlation between NETO in discrete fat depots and the sequence of fat pad depletion of Siberian hamsters as they transitioned from fat to lean after transfer from long to short day photoperiod conditions [6]. These initial studies led to a body of work that developed during the next three decades to establish the anatomic and functional role of both sympathetic and sensory innervation of white [15,16] and brown fat [17–19], the unique responses obtained from individual fat depots [20] and most recently how white and brown fat communicate to produce a coordinated metabolic and thermogenic response to physiologic challenges [19,21,22].

3. Methods of denervation

The most direct ways to test the importance of nerves in controlling adipose tissue metabolism and function is to remove the neural supply to the tissue. There are several alternate current methodologies for denervating brown or white fat depots and each of these were described in detail by Vaughan et al. [23]. One option is surgical denervation in which nerve fibers supplying a particular fat depot are physically cut. This is the most difficult and least specific procedure. It is difficult because of the necessity to locate all of the nerves that enter a specific fat depot and to dissect the nerve without damaging closely associated tissue or blood vessels. Some of these nerves may also transition through the fat depot to supply other tissue, such as adjacent skin or muscle. It is non-specific because the sympathetic and sensory nerves innervating white or brown fat depots travel together and are both destroyed when the nerve bundle is transected. Benefits are that this technique can be close to 100% effective in removing neural input and output from a fat pad and, as with chemical denervation described below, it is possible to denervate one pad in a bilateral depot and leave the other pad intact as a within animal control.

An alternative to surgical denervation is selective chemical destruction of either sympathetic or sensory neurons within a specific fat pad. 6-hydroxydopamine (6OHDA) was the first chemical agent used to produce selective destruction of sympathetic nerves. The compound is taken up into noradrenergic storage vesicles [24] through the noradrenaline re-uptake pump [25]. Oxidative damage disrupts the membrane to produce a reversible loss of sympathetic innervation [26]. Sympathectomy of a specific fat pad is achieved by making 10 to 20 small volume injections (1 or 2 μl) of 8–10 mg/ml 6OHDA across the pad [27]. Control pads receive a similar number of injections of vehicle. This is a time consuming process because the needle is held in place for a minute at the end of each injection to prevent efflux of the solution out of the tissue. However, unlike surgical denervation, sensory nerves remain intact. In a mouse study sympathetic denervation achieved with 6OHDA, measured as tissue NE content, was 60% effective 24 h after the injections. Four weeks after the injections the amount of NE per mg of tissue remained the same as at 24 h after injection [28]. Others have reported that sympathetic nerves regenerate following systemic treatment with 6OHDA. Thureson-Klein et al. [29] reported a partial recovery of brown fat NE content within 4 weeks of subcutaneous administration of 6OHDA in rats. Regeneration of the sympathetic nerves may be a disadvantage for long-term experiments designed to test the effects of denervation, but could be an advantage if the process of reinnervation is of interest. Although the sympathetic denervation of fat depots or the whole animal with 6OHDA is reversible in adults, whole animal sympathectomy of neonates is irreversible [30]. The impact of fat specific denervation in neonates has not been investigated, but may be more stable than the process in adults.

Guanethidine has also been used to sympathectomize fat depots and the procedure has been described in detail by Demas and Bartness [31]. Technically the process is similar to that for 6OHDA in that multiple small volume injections of 10 μg/μl guanethidine are made into the tissue. The guanethidine is transported into sympathetic terminals by the noradrenaline re-uptake transporter and accumulates in the vesicles containing NE [32]. Subsequently the neurons are destroyed [33] possibly due to an autoimmune response [34]. Guanethidine depletes tissue NE content by 30–80% 2 weeks after surgery [31]. As with 6OHDA this procedure leaves sensory nerves intact, but also has the added advantage of being considered permanent [35]. In 2005 we tested guanethidine, but did not find a substantial reduction in NE content of treated fat pads [36]. Others, however, continue to report effective global [37] and tissue specific [38] sympathectomy with guanethidine.

It is also possible to selectively destroy sensory nerves in adipose tissue. Capsaicin activates vanilloid receptors [39] expressed by unmyelinated and some small diameter myelinated sensory neurons. The resulting influx of calcium and sodium has an excitotoxic effect. Sensory denervation is achieved by making multiple small volume injections of 20 μg/μl capsaicin across the extent of a fat pad [23]. Destruction of the unmyelinated sensory neurons is demonstrated by a reduction in tissue calcitonin gene related protein (CGRP) and substance P content. Capsaicin does not affect unmyelinated efferent fibers [40]. Shi et al. [41] reported that tissue CGRP was reduced by approximately 40% twelve weeks after capsaicin treatment, compared with 80% in surgically denervated pads, but the advantage of chemical sensory denervation is that sympathetic fibers remain intact.

4. Sympathetic denervation and adipocyte lipolysis

The role of the sympathetic nervous system in controlling lipolysis was reviewed in depth by Bartness et al. [42], therefore, this section will summarize the evidence with an emphasis on contributions made by experiments that involved denervation of adipose tissue. Indirect evidence for sympathetic control of adipose tissue lipolysis came from early studies with cats in which splanchnic nerves were cut only on the left side. Approximately 2 weeks after the surgery denervated fat pads from overfed or food deprived cats were larger than the intact