Effects of chronic spinal cord injury on body weight and body composition in rats fed a standard chow diet

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Abstract

The inability to maintain body weight within prescribed ranges occurs in a significant portion of the human spinal cord injury (SCI) population. Using a rodent model of long-term high thoracic (spinal level T3) spinal cord transection (TX), our aim was to identify derangements in body weight, body composition, plasma insulin, glucose tolerance, and metabolic function as measured by uncoupling protein 1 (UCP1) expression in interscapular brown adipose tissue (IBAT). Sixteen weeks after SCI, body weights of injured female rats stabilized and were significantly lower than surgical control animals. At the same time point, SCI rats had a significantly lower whole body fat:lean tissue mass ratio than controls as measured indirectly by nuclear magnetic resonance. Despite lower body weight and fat mass, the cumulative consumption of standard laboratory chow (4.0 kcal/g) and mean energy intake (kcal/day/100g of body weight) of chronic SCI rats was significantly more than controls. Glucose tolerance tests indicated a significant enhancement in glucose handling in 16 wk SCI rats which were coupled with lower serum insulin levels. The post-mortem weight of gonadal and retroperitoneal fat-pads were significantly reduced after SCI and IBAT displayed significantly lower real time polymerase chain reaction (RT-PCR) expression of UCP1 mRNA. The reduced fat mass and IBAT UCP1 mRNA expression is contraindicative of the cumulative caloric intake by the SCI rats. The prolonged post-injury loss of body weight, including fat mass, is not due to hypophagia but possibly to permanent changes in gastrointestinal transit and absorption as well as whole body homeostatic mechanisms.
Introduction

Post-injury weight loss is a common consequence of any surgical procedure or major accident including spinal cord injury (SCI). The early stages of SCI are immediately marked by reduced energy expenditure, increased catabolic rate and extensive nitrogen losses that may last from weeks to several months (29, 30, 43). Nitrogen losses following SCI are compounded by the sudden and complete disuse atrophy of muscles below the level of the injury and these losses persist despite parenteral supplementation. Parenteral nutritional calculations in human acute SCI care are largely inadequate when based upon the commonly utilized Harris-Benedict equation (29, 43). For example, this inadequacy in supplementation led to poor nutritional status in para- and quadriplegic subjects over a two-week observation period (29).

In general, clinical reports indicate that SCI persons are at risk for a lifelong inability to maintain a neutral energy balance (eg.(7), (29), (30), (47), (48)). Nutritional deficits leading to an underweight body mass in SCI present a number of risk factors to the individual in both the acute and chronic phases of injury. Low body weight increases the risk of developing infection and prolongs the recovery process from major traumatic injury (13, 14). Furthermore, insufficient subcutaneous fat mass increases the risk of developing pressure ulcers (1, 45). The presentation of recurrent pressure ulcers, in turn, is a co-morbidity that triggers pro-inflammatory cytokine release, which can exacerbate the cachexic state of the patient (8).

Our aim was to test post-injury fat and lean mass, and the resulting risk for developing either excessive loss of fat mass (underweight) or the obesity-related metabolic syndrome due to excessive adiposity, in animals with chronic T3-level spinal transection. Specifically, we analyzed the whole body fat mass in vivo with nuclear magnetic resonance, for comparison with ex vivo fat pad weights and whole body weights after high thoracic spinal transection. In
addition, we analyzed the daily caloric intake of chronic SCI animals, basal glucose, glucose tolerance, plasma insulin and leptin as well as post-mortem levels of uncoupling protein-1 (UCP1) as a marker for thermogenesis within brown adipose tissue. This latter measure served as an indirect measure of thermogenesis in both groups of animals.

Materials and Methods

All procedures were performed according to National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee at the Pennington Biomedical Research Center. Wistar female rats (n = 16; Harlan) were maintained in a temperature-controlled room on a 12:12-h light-dark cycle with unrestricted access to food and water. Animals were pair-housed until surgery, which occurred at 12 weeks of age, after which animals were housed singly and observed daily. One group of rats was randomly assigned to receive a spinal cord transection centered at T2-T3 (n=10), the remaining rats (n=6) were selected to serve as surgical controls. Body weights prior to surgery were recorded and tested to ensure that no significant weight difference existed between groups.

Surgical Procedures and Animal Care

Anesthesia for all surgical procedures was Nembutal (60mg/kg, ip, Abbott Laboratories, Chicago, Ill.). Supplemental doses of all anesthetics were given as necessary to maintain areflexia. Prior to any surgical manipulation, the animal was administered ophthalmic ointment to both eyes, buprenorphine (0.1mg/kg, ip, Reckitt Benckiser Pharmaceuticals Inc., Richmond VA) to alleviate post-operative pain, and antibiotics (Baytril, 2.5 mg/ml concentration @ 1ml/kg s.q., Bayer, Shawnee Mission KS) to reduce post-surgical infection. The T2-T3 spinal cord was exposed through a midline incision over the vertebral column after which the muscles were
detached from the vertebrae. Subsequently, the T2 spinous process was removed with fine tipped rongeurs to expose the T2-T3 spinal cord surface. The segment of the dorsal median vein that marked the boundaries of the lesion site was lightly cauterized so as to limit bleeding and the lesion site was irrigated with 2% lidocaine for 5 min. Spinal transection was performed at the rostral most T3 spinal segment with microscissors and subpial aspiration (T3 TX). Procedures for the sham animals were the same as for spinal injury except that the spinal cord and surrounding dura mater were not disturbed following laminectomy. Once the surgical procedure was complete, the muscle tissue overlying the lesion site was closed in anatomical layers with Dexon II suture and the skin closed with 9mm wound clips. Animals were administered warmed supplemental fluids (5 ml lactated Ringer’s solution) and placed in an incubation chamber maintained at 37°C until the effects of anesthesia had subsided.

Chronic care of both sham and injured animals utilized procedures described previously (6). Post-operatively, animals were kept in a warm environment and received subcutaneous supplemental fluids (5-10 cc lactated ringers), analgesics (carprofen, 5mg/kg IP, Pfizer Animal Health, Lititz, PA) once daily for three days and antibiotics (Baytril, 2.5 mg/kg) twice daily for five days after surgery. Body weights and the remaining chow weight were recorded each morning for all animals and bladder expression and cleaning of the hindquarters was performed at least twice daily in animals with transection SCI until the return of spontaneous voiding. The ventrum of sham animals was inspected daily without need for manual compression of the bladder. Once spontaneous voiding returned in rats with SCI, all animals were inspected only once daily after weighing. When necessary, animals were administered additional analgesics to minimize pain and discomfort and antibiotics for 5 days following the indication of a bladder
infection. Two rats that received a spinal transection were removed from the study after developing chronic dermatitis.

**Glucose tolerance testing and blood collection**

An intraperitoneal glucose tolerance test (IPGTT) was administered to 24 h fasted animals. Each animal \((n = 16)\) received a 2 g/kg body mass dose of 5% glucose solution intraperitoneally. Blood samples (0.3 ml) were collected from the tail of lightly restrained animals and analyzed with a commercially available glucose meter (Therasense Freestyle, Abbott Laboratories, IL). Samples were drawn 10 min before injection of glucose (fasted baseline; FB) and at 15 min intervals for 75 min after injection. The area under the GTT curve was calculated for IPGTT using the trapezium method.

**Insulin assay**

During blood collection at FB, 15, 45 and 75 min after glucose, an additional 100 µl sample was taken and stored on ice in 600 µl microcentrifuge tubes. Coagulated samples were centrifuged at 4°C (5 min at 2,100 g), blood serum was collected into fresh microcentrifuge tubes, stored (−20°C), and later analyzed for serum insulin concentration at each time point using ELISA (Ultra sensitive rat/mouse insulin ELISA kit, Crystal Chem, Downers Grove, IL).

**In vivo body composition analysis**

Awake, unfasted rats were placed into the restraint tube of a Bruker minispec LF90 time domain nuclear magnetic resonance analyzer (Bruker Optics, Billerica, MA). The restraint tube was adjusted to minimize movement by the animal without impairing respiration. The tube was placed, with the animal on a horizontally-oriented axis, into the LF90 and fat mass, fat-free mass and fluid was analyzed in triplicate. The entire length of the triplicate analysis took 4 min, after which the rat was removed from the restraint tube and returned to the home cage.
**BBB locomotor rating**

Locomotor performance was assessed by open field locomotion using the standard “BBB” locomotor rating scale (5). Tests were administered at 72hr following surgery, and at once a week intervals for the duration of the experiment.

**Post mortem tissue analysis & Real-Time Polymerase Chain Reaction (PCR)**

Animals were rapidly euthanized by decapitation. The interscapular brown adipose tissue (IBAT), and bilateral retroperitoneal and genital fat pads were removed and weighed. The IBAT was immediately placed into a polyethylene centrifuge tube, frozen on dry ice, stored (~80°C), and later analyzed for uncoupling protein-1 (UCP1) mRNA using real time-PCR.

RNA was isolated using Tri-Reagent (Molecular Research Ctr, Cincinnati, OH USA) and RNeasy Minikit procedures (Qiagen, Valencia, CA USA) and based on Primeaux et al. (42). Briefly, fresh tissue was homogenized in Tri-Reagent using a motorized tissue homogenizer, chloroform was added to lysate, and the mixture was centrifuged in phase lock tubes to separate RNA. Ethanol was added to the upper aqueous phase, which was filtered by centrifugation. Following several washes, the samples were subjected to an elution step using RNAase-free water. Reverse transcription (RT) was conducted using the High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA). For RT, 2µg of RNA from each sample was added to random primers (10x), dNTP (25x), MultiScribe Reverse Transcriptase (50U/µl) and RT buffer (10x) and incubated in a thermal cycler (PTC-100, MJ Research, Inc. Watertown, MA, USA) for 10 min at 27º C, then for 120 min at 37º C. Primers for UCP1 were taken from White et al., (49). Primers for cyclophilin were designed using Primer Express (Applied Biosystems, Foster City, CA, USA). The following primers were used for UCP1: 5’-GGACCTACAATGCTTACAGAGTTATAGC-3’ and 5’-TCGTCCCTTCCACAGTGTTG-3’

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and for cyclophilin: 5’-CCCACCGTGTTCTTCGACAT-3’ and 5’-CTGTCTTTGGAACCTTTGTCTGCAA-3’. For Real-Time PCR, SYBR Green 2x Master Mix (Applied Biosystems), forward and reverse primers (10M), and RT product (10ng) were added to a 384 well plate. The cycling parameters consisted of an initial 2 min incubation at 50º C, followed by 10 min at 95º C, then 15 sec at 95 ºC, and a 1 min annealing step at 60º C (40 cycles). A dissociation step (15 sec at 95C) was added following 40 cycles to determine specificity of primers. In this assay, the dissociation step confirmed the absence of non-specific amplifications. Quantity of UCP1 mRNA was based on a standard curve and normalized to cyclophilin RNA (ABI Prism 7900 Sequence Detection System, Applied Biosystems).

Statistical analysis was performed using SPSS for Windows (SPSS Inc, Chicago, IL). Data for body weight change between groups and over time was analyzed by two-way ANOVA. Data for BBB was analyzed by one-way repeated measures ANOVA, followed by Tukey post-hoc test. Caloric intake, basal fasted blood glucose, insulin, glucose tolerance curves, NMR body composition, fat pad, brown adipose and UCP1 expression were analyzed by t-test. Significance was set at \( p<0.05. \)

**Results**

**Assessment of successful spinalization procedure:**

The BBB locomotor score of the control animals was not impaired following surgery and remained at the maximum possible score of 21 (Fig. 1). In the T3 TX rats, hind-limb function was limited to single hind-limb joint movement immediately after surgery. At day 63 post-injury, the locomotor score for these rats reached a plateau of 5.5 ± 0.9, which represents extensive range of movement in two joints and slight movement in a third. The difference in locomotor
score was significantly different between sham and T3 TX groups for all test days (F(1,12)=205.475, p<0.05).

**SCI changes ability to maintain body weight:**

Daily body weights from all experimental animals are plotted in weekly intervals as change in weight from pre-operative weight (Fig. 2). Surgical control rats receiving only a laminectomy (n=6) displayed a significant decrease (F(18,240)=7.546, p<0.05) in weight of -6.0 ± 4.86g 1 day after surgery. Control rats returned to pre-operative levels by day 14 (2.16 ± 4.79g difference from pre-op, p<0.05) and had significant weight gains thereafter. The body weight of T3 TX rats (n=8) decreased below pre-operative levels in the first week after surgery to a difference from pre-operative levels of -30.33 ± 2.44g and remained below pre-operative levels until stabilizing at pre-operative levels at week 5 (n.s., p>0.05) and remaining constant thereafter (n.s., p>0.05). Following surgery, the body weight of T3 TX animals remained significantly lower than sham animals for the duration of the experiment (F(1,12)=21.026, p<0.05). Following the overnight (18 hr) fasting periods that preceded a basal glucose test both body weight and the rate at which either group returned to pre-fasted body weights were not significantly different between T3 TX and control animals (n.s., p>0.05).

**Caloric intake is elevated in chronic SCI animals:**

During weeks 16-18, when body weights had stabilized, the daily caloric intake of standard laboratory chow was measured (Fig. 2, gray shaded region). At the end of the two week monitoring period, the cumulative caloric intake (Fig. 3a) was significantly greater in the T3 TX group (r=2.254, df=12, p<0.05). Over the two week monitoring period, this amounted to T3 TX rats consuming 19.4% more calories than sham animals, but without a significant increase in body weight (p>0.05). The mean energy intake (MEI) represents the daily average of kilocalories
consumed per 100g of body weight for each week of monitored feeding. The MEI for control animals was 20.69\pm 1.0 kcal/day/100g compared to T3 TX animals which was 30.94\pm 0.91 kcal/day/100g for week 1. For week 2, the MEI was 20.26\pm 0.90 and 29.65\pm 1.60 for controls and T3 TX animals, respectively. As seen in Fig. 3b, the MEI of T3 TX animals was significantly higher for both week 1 \((t=5.64, df=12, p<0.05)\) and week 2 \((t=3.65, df=12, p<0.05)\)

**Glucose tolerance and insulin sensitivity in chronic SCI:**

Basal blood glucose levels measured 4 hours into the light cycle at one week preoperatively and post-operative weeks 1 and 16 showed no significant difference in blood glucose between groups for any time point (Table 1, \(p>0.05\)). Pre-surgical blood glucose levels during IPGTT were not significantly different between animals assigned to either surgical group (data not shown). At 1 week following surgery, there was no difference between groups in blood glucose (Fig. 4A) or in the area under the curve (AUC) of the IPGTT (Fig. 4C, \(p>0.05\)). During the IPGTT test administered at 16 weeks after surgery, the T3 TX rats displayed a lower blood glucose level by the 30 min time point that remained lower throughout the remainder of the testing period (Fig. 4B). Subsequently, the AUC was significantly lower in the T3 TX rats \((t=2.55, df=12, p<0.05; \text{Fig} \ 4C)\). Basal insulin levels did not differ in the T3 TX rats compared to sham rats \((t=1.94, df=12, p>0.05)\). The insulin response to IPGTT was lower at all time points in the T3 TX rats (Fig. 5A) and the insulin AUC for the entire sampling period was significantly lower \((t=3.13, df=12, p<0.05; \text{Fig} \ 5B)\).

**Body composition and UCP1 RNA expression after SCI:**

The *in vivo* measure of lean tissue mass was significantly lower in T3 TX rats as measured by NMR at the conclusion of monitoring food intake \((t=5.54, df=12, p<0.05; \text{Fig} \ 6a)\). When calculated as the percentage of the whole body weight, lean mass in T3 TX animals
(68.01±1.63%) was not different from control animals (67.00±1.28%, p>0.05). The corresponding ratio of fat mass to lean tissue was even further reduced in T3 TX animals compared to controls (t= 4.40, df=12, p<0.05; Fig. 6b). Upon sacrifice, animals with T3 TX had significantly lower retroperitoneal-fat pad (t= 3.82, df=12, p<0.05) and gonadal-fat pad (t= 3.34, df=12, p<0.05) weights (Fig. 6c) while IBAT weight remained unchanged (p>0.05). Lastly, the expression level of UCP1 mRNA in the IBAT from rats in the T3 TX group (0.55 ± 0.06, mean+SEM, arbitrary units) was significantly lower than that of surgical controls (1.09 ± 0.08, mean+SEM, arbitrary units ; t=5.91, df=12, p<0.05).

**Discussion**

In the present study we demonstrate that 1) chronic low body weight, as is seen in a subset of the human SCI population, occurs in a rodent model of chronic SCI; 2) despite an increase in cumulative caloric intake in chronic SCI, low body weight persists in injured animals 3) white adipose fat mass is lower in SCI; 4) basal glucose levels are unchanged, yet glucose tolerance is significantly enhanced in chronic T3 SCI animals; 5) basal insulin levels are lower in chronic T3 SCI animals; 6) thermogenesis, as measured by UCP1 RNA, was lower in SCI animals. These data provide the first direct evidence that high thoracic SCI triggered a prolonged post-injury loss of body weight, including fat mass, which is not due to hypophagia or an increase in sympathetically mediated thermogenesis but exists despite significant hyperphagia by injured animals.

Our conclusions are based upon the following observations. Animals in our injury model received a spinal transection at the third thoracic spinal segment (T3 TX) and were maintained for 18 weeks post-injury. Transection at spinal T3 produced a permanent paraplegia in laboratory
rats and segmentally mediated locomotor reflexes were consistently observed. While locomotor recovery was not the focus of the present experiment, locomotor scores were collected as a measure of spontaneous muscle activity and movement below the level of the lesion and as a measure of lesion completeness. The BBB scores provide a common index of motor paralysis for comparison despite the inherent complexity of assessing locomotor function following SCI which has recently been addressed (36). In the present group of transected rats, BBB score was greater than in previously published data which centered transection at lower thoracic levels (6, 35). However, the animals with T3-level injury displayed qualitatively greater trunk instability and diminished fore-limb ambulation than that which is typically observed in the more common T9-level injury. Though incapable of weight support, extensive movement of all three hind limb joints was observed in our animals. The residual motor activity in the hind limbs of T3 transected animals may be sufficient enough to merit consideration in future experiments regarding the, albeit diminished, metabolic requirements of the muscle mass below the lesion.

Our weekly body weight data demonstrate that the weight disparity between SCI and non-SCI rats continued to increase throughout the experiment. This reflected the rate of increase in the non-SCI control animals, as the SCI body weights were not significantly different from pre-injury levels beginning 5 weeks post-operatively. Our observations suggest that SCI rats defend a set body weight. Specifically, we observed that SCI rats that were fasted overnight for basal glucose testing returned to pre-fasted body weight at the same rate as similarly fasted sham controls. Thus, while SCI rats maintained a lower body weight, they were capable of rapidly compensating for reduced energy intake imposed by fasting. This effect is consistent with previous reports that fasting-induced weight losses in neurally intact animals are quickly reversed once feeding is re-established (26).
Evidence from our data plotting the cumulative caloric intake of T3 TX and control animals over a two week period, indicates that the long-term injured animals were consuming a greater number of calories than controls. Post-injury muscle atrophy demonstrated previously (20, 24, 34, 44) and confirmed in our own NMR data that is coupled with a reduction in energy expenditure (31) would predict a commensurate reduction in ad lib feeding; however, during our monitoring of feeding we observed the opposite. This increased caloric consumption was more clearly evident in the mean energy intake since long-term injured animals were consuming more calories despite a lower body weight. In separate animal studies from our laboratory monitoring acute-SCI rats, the caloric intake from ad lib feeding in T3 transected rats returns to levels comparable to surgical shams within one week after surgery. As with our present data, body weight in these acute SCI animals remained below pre-injury levels through the end of the 3 week observation period, and was 20% below the cohort of surgical controls (Holmes, unpublished observations).

In the present experiment, we were unable to determine if the observed cumulative caloric intake reflected a continuing attempt to replenish diminished energy stores, or the defense of a new body weight set point that resulted from prolonged hypermetabolism. The repeated application of core temperature recording and indirect calorimetry to monitor the metabolic status of animals during the post-injury period would resolve questions about a prolonged hypermetabolic state. However, clinical evidence from the SCI population (29, 30, 43) does not favor the hypothesis of hypermetabolism for any time point over the course of this study. Furthermore, changes in core body and surface temperature have been recorded in a rat T4 transection model of SCI (32) in which core temperature dropped the day following surgery and stabilized within 3 days at a lower basal level. Those authors concluded that the resolution of
spinal shock, coupled with increased IBAT thermogenesis, led to the partial restoration of core temperature. The elevation in tail surface temperature was a likely cause of the reduced core temperature since blood flow to the tail is a major thermoregulatory mechanism in the rat (17, 39). Loss of supraspinal control over sympathetic vasoconstriction (see 19 for review) is the likely mechanism for the chronic elevation in peripheral temperatures following spinal transection (32) and not thermogenesis.

The compensatory role of IBAT thermogenesis proposed by Laird and colleagues (32) for their study remains plausible since the sympathetic preganglionic innervation of the stellate ganglion, which innervates the IBAT, is located within the mid- to upper-thoracic spinal cord (11). The injury model in that study (32) may have spared a sufficient number of preganglionic neurons to permit compensatory IBAT activation. However, without some measure of activation of IBAT tissue, the conclusions of compensatory IBAT thermogenesis following any model of mid- to upper-thoracic SCI remains speculative. One such measure involves uncoupling protein 1 (UCP1) expression in IBAT tissue since UCP1 is the mechanism responsible for thermogenesis in IBAT (reviewed in 2). Our observed reduction in IBAT UCP1 expression after chronic SCI suggests that IBAT-mediated thermogenesis was severely compromised. This most likely occurred from the moment of injury in that the majority of sympathetic preganglionic neurons are at, or below, the level of our T3 injury. It should be noted that IBAT ablation in otherwise neurally intact animals induces obesity due to decreased thermogenesis (25). Ultimately, the proposed changes in sympathetic outflow to IBAT after acute and chronic SCI require confirmation through direct electrophysiological testing of sympathetic neural activity.

Our measures of glucose tolerance and insulin sensitivity suggest highly efficient carbohydrate homeostasis which would be expected from lean subjects (18, 23). Our analysis of
both in-vivo body composition at 16 weeks after surgery and post-mortem weights of gonadal and retroperitoneal fat pads indicated that the fat mass of chronic SCI rats was significantly reduced. When available carbohydrates do not meet energy demand, triglycerides stored within the white adipose tissue are converted to free fatty acids and fat mass can rapidly deplete during an imposed fasting period (4). Surprisingly, our observed reduction in the fat mass of SCI rats occurred despite a higher cumulative caloric intake. In neurally intact animals, lipid mobilization in white adipose tissue is mediated by the sympathetic innervation of white fat (3) while denervation of white fat diminishes fat mobilization (21, 46). Fasting increases both noradrenaline turnover (37) and noradrenergic innervation of white adipose tissue (22). Since sympathetic outflow to white fat would presumably be diminished after SCI, it would be expected that white adipose tissue would increase in size after injury, but such was not the case.

Accidental trauma, including neurotrauma, and surgical procedures are considered clinically significant stress events. Both short-and long-term effects of single or repeated restraint stress on food intake, body weight and body composition have been reported in rodents (27, 28) whereby adult rats exposed to repeated restraint stress show a transient reduction in food intake, coupled with a prolonged reduction in body weight (28). The stress-related neuropeptide, corticotropin-releasing factor (CRF), has been shown to centrally influence in vivo gastrointestinal function (33, 38) which prompted Harris and co-workers to suggest that CRF-mediated reduction in gastrointestinal motility and activation of the hypothalamic-pituitary-adrenal axis initiated the short term hypophagia following restraint stress. Other studies suggest that repeated restraint stress leads to an increase in the intake of highly palatable foods or “comfort foods” and an increase in circulating corticosterone (15, 16, 40). In rats with SCI, serum corticosterone levels are increased over a 28 day period (41), which may explain, at least
in part, why rats with SCI exhibit a long-term increase in food consumption. However, the consumption of “comfort foods” in our paradigm was not directly examined. We believe that the post-SCI effect of chronic stress hormones on the complex interplay between body composition, gastric reflex function and feeding behavior may be important to our observations and remains to be systematically investigated.

In the chronic stages of SCI, muscle atrophy inherent to long-term immobility leads to a maintained reduction in resting energy expenditure (7, 9, 10). Although the injured animals became quite well adapted to fore limb locomotion, they clearly exhibited disuse atrophy of the musculature innervated below the T3 lesion and are very likely to have experienced a concomitant reduction in resting energy expenditure. This reduction in resting energy expenditure is considered a causative factor in the prevalence of increased adiposity in a majority of the human SCI population (47, 48) and requires further investigation in our model. Given the broad variability in the human cafeteria-style diet, compared with the strict standard chow diet of the typical laboratory animal, it is very likely that dietary composition is responsible for the contrasting alterations in body composition seen clinically and experimentally. While adjustments in post-injury dietary intake are often prescribed for the clinical SCI population that are at risk for obesity (12), the form and the magnitude of these adjustments are often based upon guidelines for the able-bodied population. Potential changes in alimentary absorption of nutrients as well as physiological responses to macronutrients may necessitate dietary guidelines that are specific to the SCI population. Without fully developed animal models, translational research leading to appropriate health and nutritional management strategies remain under-developed for the SCI population.
Acknowledgements

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Table 1. *Basal fasted glucose (mg/dl) in control and spinally transected rats*

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<th>Sham</th>
<th>T3 TX</th>
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<tr>
<td>Pre operative</td>
<td>61.0 ± 4.4</td>
<td>63.8 ± 2.4</td>
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Values are means ± SE.
Figure 1.

The mean BBB locomotor rating scores (± SEM) of rats tested prior to surgery (denoted as week 0) through 18 weeks following surgery. Locomotor scores were significantly lower in the spinal cord injury group (* p<0.05).

Figure 2.

Change in body weight after SCI expressed as the difference from pre-injury weight. Control animals (SHAM) did not lose weight following surgery while spinalized rats (T3 TX) displayed a significant weight reduction immediately after surgery and did not return to pre-operative weight until 5 weeks after surgery (* p<0.05). The weekly difference in weight between groups was significantly different throughout the entire course of the study. Gray shaded region denotes the period during which nightly food consumption was measured.

Figure 3.

Effect of chronic injury on the caloric intake of standard laboratory chow (Purina #5001). A. Animals with T3 TX consumed a greater nightly number of calories. This difference in caloric intake was significantly different from control animals by day 14 (* p<0.05). B. Daily mean energy intake, calculated as kcal consumed/day/100g of body weight (BW) for each week that food intake was measured, is significantly higher in long-term SCI rats compared to controls (* p<0.05).

Figure 4.

Effect of chronic injury on the IPGTT serum glucose levels at baseline (B) immediately before glucose injection (2mg/kg IP) and at 15 minute intervals after injection. A) At 1 week post-SCI, animals with T3 TX did not show any difference in glucose tolerance when compared to control animals (p<0.05). B) At 16 weeks post-SCI, glucose clearance was more rapid in the T3 TX animals. C) Glucose clearance was significantly enhanced (* p<0.05) in T3 TX animals as measured by area under the curve (AUC) for the entire glucose tolerance test.
Figure 5.

Effect of chronic injury on the IPGTT serum insulin levels in animals at 16 weeks post-SCI. A) Animals with T3 TX had lower serum insulin values when compared to control animals. B) Serum insulin was significantly lower (*p<0.05) in T3 TX animals as measured by area under the curve (AUC) for the entire glucose tolerance test.

Figure 6.

Effect of chronic injury on body composition as determined by in vivo NMR, and the post-mortem tissue weight of intrascapular brown adipose tissue (IBAT), gonadal white adipose fat pad (gWAT) or retroperitoneal white adipose fat pad (rWAT). A. The lean tissue mass (LM) as determined by in vivo NMR is significantly reduced in T3 TX animals compared to shams. B. The ratio of fat mass to lean mass (FM/LM) as determined by in vivo NMR is significantly lower in animals at 16 weeks post-SCI. C. Both post-mortem gWAT and rWAT were significantly smaller in animals with T3 TX than controls, the difference in IBAT weights did not reach statistical significance. (*p<0.05).
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Days: 1 2 3 4 5 6 7 8 9 10 11 12 13 14
Cumulative Intake (kCal)

Week 1 Week 2
Mean Energy Intake (kcal/day/100g BW)

A

B

Sham
T3 TX

* *
A

[Graph showing Raw Insulin (ng/ml) over time (min) for Sham and T3 TX groups.]

B

[Bar graph showing Insulin AUC for Sham and TX groups, with a asterisk indicating a significant difference.]