Prominent pancreatic endocrinopathy and altered control of food intake disrupt energy homeostasis in prion diseases

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Abstract

Prion diseases are fatal neurodegenerative diseases that can induce endocrinopathies. The basis of altered endocrine function in prion diseases is not well understood, and the purpose of this study was to investigate the spatiotemporal relationship between energy homeostasis and prion infection in hamsters inoculated with either the 139H strain of scrapie agent, which induces preclinical weight gain, or the HY strain of transmissible mink encephalopathy (TME), which induces clinical weight loss. Temporal changes in body weight, feed, and water intake were measured as well as both non-fasted and fasted concentrations of serum glucose, insulin, glucagon, β -ketones, and leptin. In 139H scrapie-infected hamsters, polydipsia, hyperphagia, non-fasted hyperinsulinemia with hyperglycemia, and fasted hyperleptinemia were found at preclinical stages and are consistent with an anabolic syndrome that has similarities to type II diabetes mellitus and/or metabolic

syndrome X. In HY TME-infected hamsters, hypodipsia, hypersecretion of glucagon (in both non-fasted and fasted states), increased fasted β -ketones, fasted hypoglycemia, and suppressed non-fasted leptin concentrations were found while feed intake was normal. These findings suggest a severe catabolic syndrome in HY TME infection mediated by chronic increases in glucagon secretion. In both models, alterations of pancreatic endocrine function were not associated with PrP^{Sc} deposition in the pancreas. The results indicate that prominent endocrinopathy underlies alterations in body weight, pancreatic endocrine function, and intake of food. The prion-induced alterations of energy homeostasis in 139H scrapie- or HY TME-infected hamsters could occur within areas of the hypothalamus that control food satiety and/or within autonomic centers that provide neural outflow to the pancreas.

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Introduction

A common hallmark of prion diseases is central and peripheral accumulation of a transmissible pathogenic isoform of a host-encoded prion protein that is partially resistant to proteinase-K degradation (known as PrP^{Sc}; Prusiner 1987). Deposition of PrP^{Sc} in the brain leads to neurodegenerative changes characterized by a non-inflammatory spongiform encephalopathy (for reviews, see Heikenwalder *et al.* 2007). Although progressive neurological symptoms are typical of the prion diseases, disruption of endocrine homeostasis has been observed in many instances, including those that have either an acquired, spontaneous, or familial etiology. For example, in scrapie, a prion disease of sheep and goats, hypersecretion of cortisol (Schelcher *et al.* 1999, Gayrard *et al.* 2000), growth hormone (Viguie *et al.* 2004), and insulin-like growth factors (Busiguina *et al.* 2000) have been observed, as well as altered binding capacity of cortisol-binding globulin (Gayrard *et al.* 2000). Endocrine alterations in hamsters infected with the 139H strain of the scrapie agent (hereafter, 139H scrapie) have also been reported (Carp *et al.* 1989, Ye *et al.* 1994a,b, 1997, Ye & Carp 1995; for reviews, see Carp *et al.* 1990, 1994) including

obesity, pronounced hyperinsulinemia, altered glucose tolerance and/or insulin resistance (Carp *et al.* 1989, 1990, Srinivasappa *et al.* 1989). In mouse models of prion disease, endocrine alterations range from hyperinsulinemia and adrenal-dependent obesity to loss of body weight and reduced adiposity (Carp *et al.* 1984, 1993, Kim *et al.* 1987, 1988). In fact, the loss of body weight and adiposity is common in several naturally occurring prion diseases, including sheep and goats with scrapie (Capucchio *et al.* 2001), in wild ungulates with chronic wasting disease (Miller & Williams 2002; for review see Salman 2003), and in humans with either fatal familial insomnia or Creutzfeldt–Jakob disease (Collinge 2001).

The underlying mechanisms for prion-induced alterations in endocrine homeostasis remain unknown. Evidence from both natural and experimental prion diseases suggest that peripheral endocrine glands (Ye & Carp 1995, 1996, Sigurdson *et al.* 2001), the hypothalamus (DeArmond *et al.* 1993), and the brain (Sigurdson *et al.* 2001) demonstrate varying degrees of PrP^{Sc} deposition and/or disease-related pathology by the time neurological symptoms have progressed. However, the spatiotemporal relationship between sites of prion infection and altered endocrine homeostasis has not been established. Because

prion-induced endocrinopathies appear to be associated with control of body weight/adiposity, we hypothesized that energy homeostasis, in general, is subject to prion-induced alteration. We hypothesized, as have others (Ye *et al.* 1994a,b, 1997), that there are two potential explanations for prion-induced alteration in energy homeostasis: one that involves direct PrP^{Sc} infection of peripheral endocrine tissues resulting in altered endocrine gland function, whereas the other involves PrP^{Sc} infection of central neuroendocrine tissue and/or the brain stem, resulting in alteration of central mechanisms governing endocrine feedback and/or autonomic regulation of endocrine glands.

To test the hypotheses that 1) prion diseases cause alterations in energy homeostasis and 2) direct PrP^{Sc} infection of the pancreas results in alteration of its endocrine function, we used two well-characterized hamster models of prion disease. The 139H scrapie agent causes preclinical obesity in hamsters (Carp *et al.* 1990, Hecker *et al.* 1992) and has an incubation period (i.e., the time to clinical disease) that ranges from 20 to 30 weeks (Kimberlin *et al.* 1989, Hecker *et al.* 1992, Prusiner 1998). The HY strain of the transmissible mink encephalopathic agent (HY TME) causes wasting at the later stages of clinical disease and has an incubation period that ranges from 8.5 to 9 weeks (Bessen & Marsh 1992, Bartz *et al.* 2000, 2005). In order to determine whether prion-induced alteration of pancreatic function is related to deposition of PrP^{Sc} within the pancreas, we measured fasted and non-fasted concentrations of insulin and glucagon and used western blot analysis to temporally monitor PrP^{Sc} infection of the pancreas. Our second objective was to determine whether prion-strain-specific alterations in body weight are accompanied by changes in feed intake, concentrations of hormones known to alter feed intake, and metabolites indicative of energy availability. To achieve this objective, we quantified both fasted and non-fasted concentrations of serum leptin, glucose, and β -ketones throughout disease progression within each prion strain.

Materials and Methods

Animals

Animals used in this study were 6-week-old male Syrian hamsters (*Mesocricetus auratus*) purchased from Simonsen Laboratories Inc. (Gilroy, CA, USA). Hamsters were habituated to our climate-controlled animal facility (22–24 °C, 12 h light:12 h darkness, light onset at 0700 h) for at least 7 days before initiation of the experimentation. Hamsters were allowed to feed *ad libitum* (Lab chow, Prolab RMH 3000; PMI Feeds, St. Louis, MO, USA) and water except for fasted experiments in which food was removed for ~17 h. On collection days, the terminal sampling procedures were conducted between 0900 and 1100 h. All procedures involving hamsters complied with the Guide for the Care and Use of Laboratory Animals and were evaluated and approved by the Montana State University IACUC committee.

Treatments

At the initiation of each trial (see below), hamsters were i.c. inoculated with 25 μ l of 1% brain homogenates (containing $10^{5 \times 8}$ median lethal doses) from either 139H scrapie- or HY TME-infected hamsters. These inocula were obtained previously from the hamsters that had displayed clinical prion disease prior to termination. Control hamsters (hereafter, mock hamsters) received i.c. inoculation with 25 μ l of 1% brain homogenate from normal hamsters. Following inoculations, all hamsters were observed thrice per week for clinical symptoms of prion disease. Clinical prion disease was defined as hypersensitivity in response to touch and sound, tremor of the head and shoulders, and ataxia.

Fasted and non-fasted trials and data collection

In order to obtain representative samples for each prion strain, we chose to collect terminal tissue and blood samples throughout the different incubation periods. We arbitrarily chose 25, 50, and 75% of the incubation period as well as the predicted onset of clinical disease in 139H scrapie-infected hamsters. These collection points were estimated to be 5, 10, 15, and 20 weeks p.i. based upon previous research (Kimberlin *et al.* 1989, Hecker *et al.* 1992, Prusiner 1998). We chose to take more frequent samples of hamsters inoculated with the HY TME strain since neurological symptoms of prion disease develop more rapidly compared with hamsters inoculated with other strains (Bessen & Marsh 1992). We arbitrarily chose to collect samples at 20, 40, 60, and 80% of the incubation period as well as the predicted onset of clinical disease. These collection points were estimated to be 2, 4, 6, 8, and 10 weeks p.i. based on previous research (Bessen & Marsh 1992, Bartz *et al.* 2005). At each of the predetermined collection points, a cohort (i.e., sex, time, and age matched) group of mock hamsters was also terminated and sampled in order to provide the comparative data.

In order to obtain enough serum for temporal analyses of changes in hormone/metabolite levels and to evaluate the effects of fasting on endocrine homeostasis, we conducted three different trials (Trial I, Trial II, and Trial III) on either fasted or non-fasted hamsters. In Trial I, data were collected from fasted 139H ($n=20$) and mock hamsters ($n=20$); in Trial II, from non-fasted 139H ($n=20$), HY TME ($n=25$), and mock hamsters ($n=45$); and in Trial III, from fasted HY TME ($n=15$) and mock hamsters ($n=15$). Hamsters in Trial I were assigned randomly to cages that were then assigned randomly to a particular treatment. Due to large animal-to-animal variation in initial body weights, hamsters in Trials II and III were stratified by initial body weight and then assigned randomly within strata to treatment, resulting in one large hamster, two medium-sized hamsters, and one small hamster in each cage. Neither age nor body weights were different ($P>0.98$; Fisher LSD t -test) among treatments either within or between trials on the day of inoculation.

Tissues were harvested for western blot analysis from the hamsters in Trials I and III while body weights, feed intake,

and water intake were collected weekly from the hamsters in Trial II. Changes in body weights were calculated from individual hamsters, whereas changes in feed intake, gain efficiency, and water intake were calculated from each cage of four hamsters. Gain efficiency was calculated as the ratio of total body weight gained to the amount of food consumed by each cage. On several occasions in Trial II, the number of hamsters within particular cages was diminished compared with all other cages. Therefore, feed and water intake for those cages during those specific weeks were eliminated from the data sets prior to statistical analysis.

Terminal blood and tissue collections

After hamsters were overdosed with isoflurane, blood was harvested via cardiac puncture, collected into sterile clot activator tubes (BD Sciences, Franklin Lakes, NJ, USA), and stored overnight at 2 °C. After blood collection, the brain, pituitary gland, submandibular lymph nodes, and pancreas were immediately removed, frozen on dry ice, and subsequently stored at –80 °C until western blot analysis (Trials I and III). Blood tubes were subsequently transported to the laboratory on wet ice and then centrifuged at 4 °C for 30 min at 1800 *g* (Sorvall-RT6000B, Thermo Electron Corp., Asheville, NC, USA). Serum was collected, vortexed, aliquoted (to eliminate freeze-thaw cycles), and stored at –20 °C until the analyses for hormones and metabolites.

Glucose tolerance tests – 139H scrapie-infected hamsters

In order to evaluate obesity-related changes in glucose tolerance of 139H scrapie-infected hamsters, we conducted glucose tolerance tests on six hamsters. After the hamsters were fasted for ~17 h, tests were conducted between 0930 and 1130 h (lights on at 0700 h, 12 h light:12 h darkness). Hamsters had been i.c. inoculated 23 weeks earlier with either normal hamster brain (mock) or 139H brain homogenate. All three 139H scrapie-infected hamsters displayed clinical signs of prion disease (severe motor dysfunction and ataxia) at the time of glucose tolerance tests. Hamsters were anesthetized with ketamine (100 mg/kg body weight (BW), i.p.) and kept warm on a heating pad throughout the tests. After a baseline blood sample (0.5 ml) was obtained, glucose was administered intraperitoneally at a dose of 2000 mg/kg BW. Blood samples (0.5 ml) were then obtained at 5, 10, 20, 40, and 60 min post-injection from either the saphenous vein or toenail clipping, collected into sterile capillary spin tubes, and immediately placed at 4 °C. After tests were complete, blood was immediately processed for serum recovery and then frozen at –20 °C until the analysis for glucose and insulin.

Tissue preparation and purification of PrP^{Sc} for gel electrophoresis

PrP^{Sc} purification of tissue was performed prior to western blot analysis as previously described (Bartz *et al.* 2003, 2005) with modifications based on the work of Herzog *et al.*

(2005). Briefly, tissues were weighed, minced with a razor blade, and mixed with digestion buffer (25 mM HEPES (pH 7.2), 0.3 M sucrose, Liberase Blendzyme 2 (F Hoffmann–La Roche Ltd – Diagnostics Division, Basel, Switzerland), and trypsin inhibitor from soybeans (Sigma–Aldrich Corp.)) to a 10% (wt/vol.) mixture and then incubated for 60 min at 37 °C with constant agitating. The mixtures were then passed through a series of needles until completely homogenized and protease activity was stopped by adding complete mini-protease inhibitor (F Hoffmann–La Roche Ltd – Diagnostics Division) and setting samples on ice for 10 min. An aliquot (100–750 µl, depending on tissue) of each homogenate was mixed with an equal volume of buffer (20% (wt/vol.) *N*-lauroylsarcosine in 10 mM Tris–HCl (pH 7.5)) followed by several rounds of ultracentrifugation as described by Bartz *et al.* (2003, 2005). The second pellet from these spins was resuspended in a Tris-buffered saline and subjected to sonication using a cup horn sonicator (Fisher Scientific, Atlanta, GA, USA). In order to degrade the normal prion protein (PrP^C), Proteinase-K (F Hoffmann–La Roche Ltd – Diagnostics Division) was added to a final concentration of 0.4 U/ml and the suspension was incubated at 37 °C for 30 min with constant agitating. Afterwards, phenylmethyl-sulfonyl fluoride was added to reach a concentration of 1 mM. The third pellet was resuspended in a NuPAGE denaturing buffer and reducing agent and then stored overnight at –20 °C until gel electrophoresis the following day.

Gel electrophoresis and western blot analysis for PrP immunoreactivity

Tissue homogenates enriched for PrP^{Sc} were subjected to gel electrophoresis for detection of PrP immunoreactivity. Briefly, samples were boiled for 10 min, allowed to come to room temperature, and centrifuged in a microcentrifuge (Eppendorf 5415 D, Eppendorf Corporation, Westbury, NY, USA) at 16 110 *g* at room temperature for 10 min. Samples were loaded onto 12% Bis–Tris NuPAGE gels (Invitrogen) and ran at 100 V in morpholinepropanesulfonic acid buffer for ~3.25 h. Proteins were then transferred at 30 V for 1 h onto a polyvinylidene difluoride membrane, which were then subjected to a 1 h wash in a 5% (wt/vol.) Blotto blocking buffer (Bio-Rad Laboratories) and then incubated overnight at room temperature with a mouse anti-PrP 3F4 monoclonal antibody (a gift of Victoria Lawson, NIH Rocky Mountain Labs, Hamilton, MT, USA, described by Kascsak *et al.* 1987) at a dilution of 1:40 000 in the above blocking buffer. Following several washes in Tris-buffered saline with Tween–20, blots were subjected to chemiluminescent detection. The detection system included incubation with an anti-mouse immunoglobulin G alkaline phosphatase conjugate (Promega) at a dilution of 1:15 000 in the Blotto buffer. Blots were developed using CDP-Star substrate (Applied Biosystems, Foster City, CA, USA) and imaged with a Kodak Image Station 2000MM and Kodak 1-D software (Eastman Kodak Company).

Hormone and metabolite analysis

Glucose concentrations were determined in duplicate 10 µl aliquots by an end point enzymatic assay using a commercially available glucose determination kit (Infinity Glucose Hexokinase liquid reagent, ThermoElectron Corporation, Waltham, MA, USA). Standard curves were developed using reagent grade D-glucose (Sigma-Aldrich Corp.). Percent recoveries averaged $98 \pm 2\%$ when the middle glucose standard (80 mg/dl) was spiked into different volumes of hamster serum. The sensitivity of the assay (absorbance of reagent blank -2 S.D. of the lowest standard value) averaged 4 mg/dl, and the intra- and inter-assay coefficients of variation were 4.4 and 7.4% for 10 µl of a serum pool from fasted hamsters that had an average absorbance of 0.637 ± 0.014 units when read on a spectrophotometer at 340 nm.

Insulin concentrations were determined in duplicate 100 µl aliquots by RIA using a commercially available rat insulin kit (Linco Research, St Louis, MO, USA) validated for use in hamsters in our laboratory. Parallelism between serial dilutions of pooled hamster samples and rat insulin standards was confirmed. Percent recoveries averaged $98 \pm 3\%$ when the middle rat insulin standard (0.5 ng/ml) was spiked into different volumes of pooled hamster serum. The sensitivity of the assay (100% bound concentration -2 S.D. of maximum binding) averaged 110 pg/ml, and the intra- and inter-assay coefficients of variation were 4.2 and 6.9% for 100 µl of a serum pool from fasted hamsters that inhibited binding of labeled hormone to $58 \pm 2\%$ of maximal binding.

Glucagon concentrations were determined in duplicate aliquots of serum (50 or 100 µl, depending upon fasted state) by a commercially available rat glucagon RIA kit (Linco Research) validated for use in hamsters in our laboratory. Parallelism between serial dilutions of pooled hamster samples and rat glucagon standards was confirmed. Percent recoveries averaged $99 \pm 4\%$ when the middle rat glucagon standard (100 pg/ml) was spiked into different volumes of pooled hamster serum. The sensitivity of the assay (100% bound concentration -2 S.D. of maximum binding) averaged 36 pg/ml, and the intra- and inter-assay coefficients of variation were 2.6 and 8.3% for 50 µl of a serum pool from fasted hamsters that inhibited binding of labeled hormone to $48 \pm 4\%$ of maximal binding.

The β -ketone concentrations were determined in a single 10 µl aliquot using a commercially available, hand-held glucose/ketone monitor (Precision Xtra, MediSense/Abbott laboratories). Serum was applied to the electrochemical strip, and after 30 s the sensor displayed the concentration of sample β -ketones (mmol/l). Aqueous samples of β -hydroxybutyrate (Sigma) were produced from 0.01 to 10 mmol/l, in order to determine accuracy of the sensor. Linear regression analysis showed that the meter was accurate between ~ 0.1 and 5 mmol/l ($r^2 = 0.951$). The maximum read-out value was 6 mmol/l; therefore, the meter's accuracy above this concentration could not be determined. Percent recoveries averaged $92 \pm 2\%$ when the middle ketone standard (0.6 mmol/l) was spiked into different volumes of normal hamster serum.

The lowest concentration of aqueous standard detected by the meter was 0.01 mmol/l. The intra- and inter-assay coefficients of variation were 3.6 and 9.4% for 10 µl of a serum pool from fasted hamsters that had an average readout of 1.4 ± 0.1 mmol/l.

Leptin concentrations were determined in duplicate aliquots of 10 µl serum by a commercially available rat leptin ELISA kit (Linco Research) validated for use in hamsters in our laboratory. Parallelism between serial dilutions of pooled hamster samples and rat leptin standards was confirmed. Percent recoveries averaged $94 \pm 3\%$ when the middle rat leptin standard (0.5 ng/ml) was spiked into different volumes of pooled hamster serum. The sensitivity of the assay (absorbance of reagent blank wells -2 S.D. of the lowest standard value) averaged 0.05 pg/ml, and the intra- and inter-assay coefficients of variation were 2.98 and 4.4% for 10 µl of a serum pool from fasted hamsters that resulted in a net absorbance of 0.143 ± 0.05 units when readings at 590 nm were subtracted from those at 450 nm on an ELISA microplate reader (ELx800, Bio-Tek Instruments, Inc., Winooski, VT, USA).

Statistical analysis

Weekly changes in individual body weight, cage feed intake, cage gain efficiency, and cage water intake, along with terminal serum concentrations of glucose, insulin, glucagon, β -ketones, and leptin were analyzed in separate ANOVA using the generalized linear model approach and the Statistical Analysis System (SAS v. 9.0.3., Cary, NC, USA). Based on the previous data in hamsters (Borer *et al.* 1979, Rowland 1984) showing that fasting can affect the concentrations of hormones and metabolites of interest, separate ANOVA were constructed for fasted and non-fasted hamsters. Each model included the effects of treatment (i.e. 139H scrapie, HY TME, or mock), time (i.e. weeks p.i.), and the treatment $\times$ time interaction. Data from the glucose tolerance tests were analyzed using individual animals as the experimental unit and subjected to repeated measures ANOVA using the generalized linear model approach and the Statistical Analysis System (SAS v. 9.0.3). The effects of treatment, time (i.e. time of sampling), and the treatment $\times$ time interaction were included in the model. When the *F* value indicated a significant effect ($P < 0.05$) in the main ANOVA model, least-square (ls) means procedures were used to calculate means and standard errors. Pairwise *t*-tests using Bonferroni's correction for multiple comparisons was used for statistical analysis of the means associated with significant effects. All data reported in this manuscript are lsmeans $\pm$ S.E.M.

Results

Incubation periods for 139H scrapie- and HY TME-infected hamsters

In the present study, eight out of ten hamsters (i.e., $n = 5$ from Trial I and $n = 5$ from Trial II) infected with the 139H scrapie strain and killed at 20 weeks p.i. displayed initial signs of prion disease; however, since the studies were terminated at this

time, a progression of clinical prion disease symptoms could not be verified. Therefore, we could not calculate incubation period data for 139H scrapie-infected hamsters. Previous unpublished data from our laboratory found the incubation period to be 17 ± 0.1 weeks p.i. for 139H scrapie-infected hamsters, similar to the data from Kimberlin *et al.* (1989) who found incubation periods of ~ 18 weeks p.i. However, these data are markedly different from the incubation periods reported by Hecker *et al.* (1992) and Prusiner (1998), which were 24 and 25 weeks p.i. respectively. As stated previously, the 139H scrapie-infected hamsters used for the glucose tolerance tests, which were at 23 weeks p.i., displayed clinical symptoms of prion disease including severe motor dysfunction and ataxia. The variation we and other researchers have observed in incubation periods are likely due to differences in the concentration of infectious units in the different inocula, which is known to strongly influence the time required to develop clinical disease symptoms (Hecker *et al.* 1992). To ensure that the lack of clinical prion disease in the present study was not due to lack of PrP^{Sc} deposition, we conducted PrP^{Sc} enrichment and western blot analysis of brain tissue collected from three of the 139H scrapie-infected hamsters killed at 20 weeks p.i. Of the three brains analyzed, all were found to be immunopositive for PrP^{Sc} (Table 1).

The incubation period for HY TME-infected hamsters was 9.7 ± 0.3 weeks p.i., which is in agreement with previous work (Bessen & Marsh 1992, Bartz *et al.* 2000, 2005) showing a range of incubation periods from 8.5 to 9 weeks p.i. respectively. Six out of the eight HY TME-infected hamsters killed at 10 weeks p.i. were considered clinical as they displayed severe impairments of motor coordination, head/shoulder tremors, and stimulus sensitive cloni. PrP^{Sc} enrichment and western blot analysis of the brains of three of the HY TME clinical hamsters were found to be immunopositive for PrP^{Sc} (Table 1).

As predicted, mock hamsters did not show abnormal neurological signs typical of prion disease and evidence of PrP^{Sc} deposition in any of the tissues subjected to enrichment and western blot analysis (data not shown).

Body weight, feed intake, gain efficiency, and water intake in 139H scrapie- and HY TME-infected hamsters

Body weight Changes in body weights (Fig. 1A) were not different ($P > 0.10$) among treatments for the first 8 weeks of the trial. At both 9 and 10 weeks p.i., HY TME-infected hamsters had lower ($P < 0.05$) body weight when compared with either mock or 139H scrapie-infected hamsters respectively. By 11 weeks p.i., and continuing throughout the remainder of the trial, the 139H scrapie-infected hamsters were heavier ($P < 0.05$) than the mock hamsters. These data support previous observations (Carp *et al.* 1990, Ye *et al.* 1994b, 1997, Ye & Carp 1996) in which the 139H strain of scrapie agent caused preclinical obesity in hamsters.

Feed intake Cage feed intake (Fig. 1B) did not differ ($P > 0.10$) among treatments for the first 11 weeks of the experiment. Thereafter, from 11 weeks p.i. to the end of the experiment, 139H scrapie-infected hamsters had greater ($P < 0.05$) feed intake than mock hamsters. These data are the first to show that 139H scrapie-infected hamsters display increased feed intake relative to time- and age-matched mock hamsters whereas HY TME-infected hamsters maintained normal feed intake for 10 weeks p.i.

Gain efficiency Cage gain efficiency (Fig. 1C), which was calculated from the ratio of total body weight gained within a cage to the total amount of food consumed by the cage, did not differ ($P > 0.10$) among treatments for the first 8 weeks of the experiment. At 9 weeks p.i., the 139H scrapie-infected hamsters had increased ($P < 0.01$) gain efficiency compared with either HY TME or mock hamsters, for which the gain efficiency was similar ($P > 0.10$) at this time. From 10 weeks p.i. to the end of the experiment, 139H hamsters had higher ($P < 0.05$) gain efficiency than mock hamsters. At 10 weeks p.i., HY TME-infected hamsters had diminished ($P < 0.05$) gain efficiency when compared with either mock- or 139H scrapie-infected hamsters respectively. These data suggest that

Table 1 Spatiotemporal deposition of PrP^{Sc} in central and peripheral tissues in hamsters receiving intracerebral inoculation with either 139H scrapie or HY transmissible mink encephalopathy (HY TME)^a

		139H Scrapie				HY TME			
Inoculum									
Weeks p.i.		5	10	15	20	2	4	6	10
Percentage of incubation period ^b		25%	50%	75%	100%	20%	40%	60%	100%
		Number of hamsters out of three analyzed that were immunopositive for PrP ^{Sc}							
Tissue									
mg (T.E.) ^c									
Brain	10	3	3	3	3	0	1	3	3
Pituitary	<10	0	1	3	3	0	0	0	3
SMLN	25	3	3	3	3	0	2	0	1
Pancreas	50–150	0	0	0	0	0	0	0	0

p.i., post-inoculation; SMLN, submandibular lymph nodes.

^aAll tissues from mock hamsters were found to be devoid of detectable PrP^{Sc} immunoreactivity.

^bApproximate percent of predicted incubation period that had elapsed.

^cAmount of tissue subjected to PrP^{Sc} enrichment and western blot analysis on a tissue equivalent (T.E.) basis.

139H-infected hamsters gain more body weight per unit of food consumed when compared with either mock- or HY TME-infected hamsters. Furthermore, by 10 weeks p.i., HY TME-infected hamsters fail to gain weight despite adequate food intake, resulting in negative gain efficiency.

Water intake Cage water intake (Fig. 1D) did not differ ($P > 0.10$) among treatments for the first 8 weeks of the experiment. At 9 and 10 weeks p.i., HY TME-infected hamsters had reduced ($P < 0.01$) water intake when compared with either 139H scrapie or mock hamsters respectively. From 11 weeks p.i. to the end of the experiment, 139H hamsters had higher ($P < 0.05$) water intake than mock hamsters. These data suggest that HY TME-infected hamsters develop hypodipsia whereas 139H scrapie-infected hamsters develop polydipsia.

Endocrine control of energy homeostasis in non-fasted and fasted 139H scrapie-infected hamsters

Serum glucose Non-fasted glucose concentrations did not differ ($P > 0.10$) between 139H and mock hamsters at 5

weeks p.i. At 10, 15, and 20 weeks p.i., non-fasted glucose concentrations were higher ($P < 0.01$) in 139H scrapie-infected hamsters (Fig. 2A) than mock hamsters. Fasted glucose in 139H scrapie and mock hamsters was not different ($P > 0.10$) at 5 or 10 weeks p.i., however, it was higher ($P < 0.05$) in 139H scrapie hamsters at 15 and 20 weeks p.i. respectively (Fig. 2B). These data suggest that 139H scrapie-infected hamsters develop preclinical hyperglycemia, which was most evident during the non-fasted state, which is in agreement with Srinivasappa *et al.* (1989).

Serum insulin Concentrations of non-fasted insulin did not differ ($P > 0.10$) between 139H and mock hamsters at 5 weeks p.i. Thereafter, 139H scrapie-infected hamsters had higher ($P < 0.01$) non-fasted concentrations of insulin at 10, 15, and 20 weeks p.i. respectively (Fig. 2C). Concentrations of fasted insulin did not differ ($P > 0.10$) at 5, 10, and 15 weeks p.i. between 139H and mock hamsters. However, at 20 weeks p.i., fasted insulin was higher ($P < 0.001$) in 139H scrapie-infected hamsters than in mock hamsters. The data suggest that 139H scrapie-infected hamsters develop preclinical

Figure 1 Effects of intracerebral inoculation of hamsters with 139H scrapie (square with solid line), HY TME (upside-down triangle with solid line), or normal brain homogenate (mock, open circle with dashed line) on changes in individual (A) body weight, (B) cage food intake, (C) cage gain efficiency, and (D) cage water intake. Measurements were made weekly beginning on the day of inoculation. Differences among treatments are noted within the brackets encompassing particular ranges of days post-inoculation. The number of hamsters measured within each treatment are as follows: 1) HY TME, $n=25$, with five hamsters terminated at 2, 4, 6, 8, and 10 weeks post-inoculation respectively; 2) 139H scrapie, $n=20$, with five hamsters terminated at 5, 10, 15, and 20 weeks post-inoculation respectively; and 3) mock, $n=40$, with five hamsters terminated at 2, 4, 5, 6, 8, 10, 15, and 20 weeks post-inoculation respectively. Data shown are $\text{Ismeans} \pm \text{S.E.M.}$

Figure 2 Effects of intracerebral inoculation of hamsters with 139H scrapie (black bars) or normal brain homogenate (mock, gray bars) on changes in non-fasted (A, C, E, and G) and fasted (B, D, F, and H) concentrations of serum glucose (A and B), insulin (C and D), glucagon (E and F), and β -ketones (G and H). Within each panel, bars with different letters differ at $P < 0.05$. Treatment $\times$ time interactions are presented for all variables, however, when the treatment $\times$ time interaction was not significant ($P > 0.10$), the significance level of the treatment effect is presented on the graph. Data shown are $\text{lsmeans} \pm \text{s.e.m.}$. *Significant treatment effects are associated with the following comparisons: 1) E, 135.4 ± 15.5 pg/ml (139H) $> 114.5 \pm 10$ pg/ml (mock), $P < 0.05$ and 2) F, 160 ± 4.5 pg/ml (139H) $< 179.9 \pm 7.7$ pg/ml (mock), $P < 0.05$.

hyperinsulinemia that was accentuated during the non-fasted state. These data are in agreement with several previous studies (Srinivasappa *et al.* 1989, Carp *et al.* 1990).

Serum glucagon Non-fasted glucagon concentrations did not differ ($P > 0.10$) between 139H and mock hamsters at any time point evaluated (Fig. 2E). Nevertheless, the treatment effect ($P < 0.05$) indicated that, overall, non-fasted concentrations of glucagon were higher ($P < 0.05$) in 139H scrapie-infected hamsters than in mock hamsters. Similarly, fasted 139H scrapie and mock hamsters had similar ($P > 0.10$) concentrations of glucagon at each time point (Fig. 2F), whereas the treatment effect ($P < 0.05$) indicated that overall 139H scrapie-infected hamsters had lower ($P < 0.05$) fasted glucagon than mock hamsters. These data suggest that 139H scrapie hamsters had higher than normal glucagon secretion during the non-fasted state and diminished glucagon response to fasting.

Serum β -ketones Non-fasted and fasted concentrations of β -ketones were not different ($P > 0.10$) between 139H and mock hamsters at all time points and did not change ($P > 0.10$) over time in either treatment (Fig. 2G and H). These data indicate that oxidation of fatty acids and/or liberation of β -ketones is not altered by 139H scrapie infection in hamsters.

Glucose tolerance tests At baseline, 139H scrapie-infected hamsters displayed higher levels ($P < 0.05$) of glucose when compared with mock hamsters at baseline (Fig. 3A). At 5, 10, and 20 min post-injection, glucose levels increased ($P < 0.05$) in both treatments but did not differ at these time points. However, at 40 and 60 min post-injection, 139H hamsters had higher ($P < 0.05$) glucose compared with mock hamsters. While glucose decreased ($P < 0.05$) in mock hamsters from 20 to 60 min post-injection, there was no corresponding change ($P > 0.10$) in glucose in 139H scrapie-infected hamsters. Serum insulin levels were higher ($P < 0.05$) in 139H compared with mock hamsters at all time points evaluated, including baseline, and did not change over time ($P > 0.10$). Alternatively, mock hamsters showed a temporal pattern of insulin secretion that increased ($P < 0.05$) from baseline to 20 min post-injection, followed by a decrease ($P < 0.05$) from 20 to 60 min post-injection, indicating normal glucostasis (Figure 3B).

Figure 3 Effects of intracerebral inoculation of hamsters with 139H scrapie (solid squares with a solid line) or normal brain homogenate (mock, open circles with a dashed line) on changes in serum (A) glucose and (B) insulin following i.p. injection of glucose (2000 mg/kg). Within each panel, data points with different letters differ at $P < 0.05$. Data shown are $\text{lsmeans} \pm \text{s.e.m.}$.

These results suggest that the clinical disease in 139H scrapie-infected hamsters is associated with impaired glucose tolerance, insulin resistance, and unresponsive pancreatic β cells.

Serum leptin Concentrations of non-fasted leptin did not differ ($P > 0.10$) between 139H- and mock-infected hamsters at any time point and did not change ($P > 0.10$) over time in either treatment (Fig. 5A). Nevertheless, the overall treatment effect ($P < 0.02$) indicated that non-fasted 139H scrapie-infected hamsters had lower ($P < 0.05$) concentrations of leptin than non-fasted mock hamsters. Conversely, fasted concentrations of leptin were higher ($P < 0.05$) in 139H hamsters than mock hamsters at 10, 15, and 20 weeks p.i. respectively (Fig. 5B). These data suggest that 139H scrapie infection was related to increased levels of leptin during fasting as well as overall diminished levels of leptin during the non-fasted state.

Endocrine control of energy homeostasis in non-fasted and fasted HY TME-infected hamsters

Serum glucose Non-fasted concentrations of glucose were not different ($P > 0.10$) between HY TME and mock hamsters at any time point (Fig. 4A). Nevertheless, the overall treatment effect ($P < 0.02$) indicated that non-fasted HY TME-infected hamsters had higher ($P < 0.05$) concentrations of glucose than non-fasted mock hamsters. Fasted concentrations of glucose were not different between HY TME- or mock-infected hamsters at 2 or 4 weeks p.i., but were diminished ($P < 0.05$) in HY TME-infected hamsters compared with mock hamsters at 6, 8 and 10 week p.i. respectively (Fig. 4B). Nevertheless, the overall treatment effect ($P < 0.05$) indicated that the fasted HY TME-infected hamsters had lower ($P < 0.01$) glucose concentrations than the fasted mock hamsters. These data suggest alterations in glucostasis in the HY TME-infected hamsters including increased glucose during the non-fasted state, and diminished glucose availability during fasting.

Serum insulin Non-fasted concentrations of insulin were not different ($P > 0.10$) between HY TME and mock hamsters at 2, 4, 6, or 8 weeks p.i. At 10 weeks p.i., concentrations of non-fasted insulin were higher ($P < 0.05$) in HY TME- than mock-infected hamsters (Fig. 4C). Fasted concentrations of insulin in HY TME and mock hamsters were not different ($P > 0.10$) at any time point (Fig. 4D). Nevertheless, the overall treatment effect ($P < 0.05$) showed that HY TME-infected hamsters had higher ($P < 0.02$) fasted insulin than mock hamsters. These data suggest that HY TME-infected hamsters develop non-fasted hyperinsulinemia by clinical disease.

Serum glucagon Non-fasted concentrations of glucagon were not different ($P > 0.10$) between HY TME and mock hamsters at 2, 4, and 6 weeks p.i. Non-fasted concentrations of glucagon tended ($P = 0.06$) to be higher at 8 weeks and were higher ($P < 0.01$) by 10 weeks p.i. in HY TME- than mock-infected hamsters (Fig. 4E). Fasted concentrations of glucagon were not different ($P > 0.10$) between HY TME

Figure 4 Effects of intracerebral inoculation of hamsters with HY TME (white bars) or normal brain homogenate (mock, gray bars) on changes in non-fasted (A, C, E, and G) and fasted (B, D, F, and H) concentrations of serum glucose (A and B), insulin (C and D), glucagon (E and F), and β -ketones (G and H). Within each panel, bars with different letters differ at $P < 0.05$. Treatment $\times$ time interactions are presented for all variables, however, when the treatment $\times$ time interaction was not significant ($P > 0.10$), the significance level of the treatment effect is presented on the graph. Data shown are $\text{lsmeans} \pm \text{S.E.M.}$ *Significant treatment effects are associated with the following comparisons: 1) A, $183 \pm 51 \text{ mg/dl}$ (HY TME) $> 127 \pm 16 \text{ mg/dl}$ (mock), $P < 0.05$ and 2) D, $0.84 \pm 0.17 \text{ ng/dl}$ (HY TME) $> 0.56 \pm 0.1 \text{ ng/dl}$ (mock), $P < 0.05$.

and mock hamsters at 2 and 4 weeks p.i., however, they were higher ($P < 0.04$) in HY TME-infected hamsters at 6, 8, and 10 weeks p.i. respectively than mock-infected hamsters (Fig. 4F). These data suggest that glucagon levels in HY TME-infected hamsters become chronically elevated during fasting by as early as 6 weeks p.i.

Serum β -ketones Non-fasted concentrations of β -ketones were not different ($P < 0.05$) between HY TME and mock hamsters at any time point and did not change ($P > 0.10$) over time in either treatment (Fig. 4G). Fasted concentrations of β -ketones were not different at 2 and 4 weeks p.i., but were higher ($P < 0.02$) in HY TME-infected hamsters than fasted mock hamsters at 6, 8, and 10 weeks p.i. respectively. These data suggest that HY TME-infected hamsters develop a chronic catabolic response to fasting as early as 6 weeks p.i. which continued into clinical disease.

Serum leptin Non-fasted concentrations of leptin were not different ($P > 0.10$) between HY TME and mock hamsters at 2, 4, 6, or 8 weeks p.i. (Fig. 5C) but were lower ($P < 0.01$) in HY TME- than in mock-infected hamsters at 10 weeks p.i. Fasted concentrations of leptin were not different ($P > 0.10$) between HY TME and mock hamsters at any time point and did not change ($P > 0.10$) over time in either treatment. These data suggest that HY TME infection in hamsters results in a diminished leptin response during a non-fasted state.

Analysis of PrP^{Sc} content in tissues by western blot

In order to examine whether alterations in pancreatic endocrine function were related to PrP^{Sc} deposition in the pancreas, we conducted PrP^{Sc} enrichment of pancreata from three out of five hamsters killed at each time point, namely, 5, 10, 15, and 20 weeks p.i. in both 139H scrapie and mock hamsters. PrP^{Sc} was not detected in any of the pancreata of 139H scrapie-infected hamsters. Subsequent western blot analysis of a pooled sample of pancreata from 139H scrapie-infected hamsters killed at 20 weeks p.i. again revealed a lack of PrP^{Sc} deposition (data not shown). Western blot analyses of tissues from 139H scrapie-infected hamsters revealed pre-clinical deposition of PrP^{Sc} in submandibular lymph nodes by 5 weeks p.i. and pituitary glands by 10 weeks p.i. (Table 1).

Western blot analyses of HY TME hamster pancreata also revealed a lack of pancreatic PrP^{Sc} deposition at all time points analyzed (Table 1) and similar results were obtained when samples were pooled (data not shown). Western blot analyses of other tissues from HY TME-infected hamsters revealed

PrP^{Sc} deposition in submandibular lymph nodes by 4 weeks p.i. and in pituitary glands by 8 weeks p.i. (Table 1).

Discussion

Prion-induced changes in body weight are accompanied by prion strain-specific alterations in energy homeostasis

Our analysis of 139H scrapie and HY TME in hamsters indicate that prior to the development of neurological dysfunction, PrP^{Sc} deposition causes complex, strain-specific alterations in energy homeostasis including changes in body weight and disruption of endocrine homeostasis. Importantly, the magnitude of endocrine disruption was affected by fasting status and largely depended upon the stage of infection (i.e., preclinical compared with clinical). Specifically, in the 139H scrapie model, a severe preclinical anabolic syndrome was evident and was more pronounced when hamsters were non-fasted, whereas in the HY TME model a severe catabolic syndrome developed by clinical disease and was more pronounced when hamsters were fasted. Although endocrine data associated with glucostasis are scarce from other prion disease models, sheep with clinical scrapie disease appear to also have increased concentrations of insulin and are also hyperglycemic (Viguie *et al.* 2004) although scrapie-infected sheep actually lose weight near clinical disease onset (Capucchio *et al.* 2001).

139H scrapie infection in hamsters induces chronic elevations in insulin, hyperglycemia, and impairs glucose tolerance

The anabolic syndrome that we documented in 139H scrapie-infected hamsters confirms previous observations made by Srinivasappa *et al.* (1989) and Carp *et al.* (1990) including preclinical obesity and hyperinsulinemia. However, Carp *et al.* (1990) further reported that by 18 weeks p.i., fasted 139H scrapie-infected hamsters developed hypoglycemia. This conclusion was based upon a single glucose evaluation at 2 h post-overload compared with the more intensive tests we and others have employed. Data from the glucose tolerance tests conducted by Carp *et al.* (1990) disagree with our intensive glucose tolerance tests that revealed marked hyperglycemia by 40 min post-overload in clinical 139H scrapie-infected hamsters. Similar glucose tolerance tests performed at 81 days p.i. (~12 weeks p.i.) by Srinivasappa *et al.* (1989) revealed hyperglycemia by 30 min post-overload. It is difficult to ascertain why there is a discrepancy between the Carp *et al.* (1990) study and our findings (as well as those of Srinivasappa *et al.* (1989)), but it could be related to the different methodologies employed (e.g. glucose assays). We evaluated a hand-held glucometer (similar to the one used by Carp *et al.* (1990) and found unacceptable reproducibility for analytical purposes, a general lack of precision, and an underestimation of glucose when known levels were moderately high (e.g., >160 mg/dl). Therefore, we chose an end point enzymatic assay, as did Srinivasappa *et al.* (1989), that generated consistent

Figure 5 Effects of intracerebral inoculation of hamsters with 139H scrapie (black bars, A and B), HY TME (white bars, C and D) or normal brain homogenate (mock, gray bars, A–D) on changes in non-fasted (A and C) and fasted (B and D) concentrations of serum leptin. Within each panel, bars with different letters differ at $P < 0.05$. Treatment $\times$ time interactions are presented for all variables, however, when the treatment $\times$ time interaction was not significant ($P > 0.10$), the significance level of the treatment effect is presented on the graph. Data shown are $\text{mean} \pm \text{S.E.M.}$. *Significant treatment effect is associated with the following comparison: 1) A, $11 \pm 1.5 \text{ ng/ml}$ (139H) $< 15.3 \pm 1.3 \text{ ng/ml}$ (mock), $P < 0.05$.

results over serially diluted, pooled hamster sera and in test samples spiked with very high glucose concentrations (i.e., >640 mg/dl). Overall, the sensitivity, precision and accuracy of glucose determination were vastly improved by utilizing the enzymatic method. The present studies provide strong evidence that impaired glucose tolerance, increased insulin resistance, hyperphagia, and increased gain efficiency in 139H scrapie-infected hamsters are consistent with the observed hyperglycemia. Additionally, the glucose tolerance tests suggest that pancreatic β cells are unresponsive to changes in glucose and/or are chronically stimulated to secrete insulin through a mechanism that may not be directly related to energy status. Regardless, it is our position that these specific alterations in energy homeostasis lead directly to the observed anabolic syndrome and, eventually, cause severe obesity. Importantly, the increased body weights in 139H scrapie-infected hamsters are probably not caused by an overall increase in growth rate, since femur and overall body lengths are not patently different from mock hamsters (Carp *et al.* 1990).

HY TME infection in hamsters induces hypersecretion of glucagon and alters glucose availability resulting in increased levels of serum β -ketones during fasting

We are the first group to describe endocrine alterations that underlie the catabolic syndrome in HY TME-infected hamsters. Alterations in glucostasis were evident in both fasted and non-fasted states. Specifically, modest hyperglycemia was apparent in non-fasted HY TME hamsters whereas glucose levels were diminished in the fasting state. Additional changes included non-fasted hyperinsulinemia at clinical disease in addition to generalized increases in fasting levels of insulin. The paradoxical relationship between suppressed levels of fasting glucose and increased levels of fasting insulin indicate that HY TME infection is associated with severe disturbances in pancreatic endocrine function. In addition, the endocrine and behavioral alterations that normally occur in response to fasting/catabolism, namely compensatory feeding bouts and rapid replenishment of lost energy stores (Borer *et al.* 1979, Rowland 1984) appear to also be perturbed in HY TME-infected hamsters. In this regard, our data indicate that HY TME infection in hamsters induces a catabolic state that is related to chronic increases in serum glucagon levels, suppressed availability of glucose during the fasted state and, possibly, a failure of compensatory feeding as energy stores become severely depleted. Importantly, the loss of body weight and reduced glucose availability in HY TME-infected hamsters were not the result of diminished feed intake but may be related to diminished efficiency of gain.

Are prion strain-specific alterations of pancreatic endocrine function temporally related to PrP^{Sc} deposition within the pancreas?

The western blot analyses showed that despite PrP^{Sc} deposition in some peripheral tissues, including the pituitary gland and submandibular lymph nodes, the pancreas did not have

detectable levels of PrP^{Sc} accumulation in either 139H or HY TME infection. This was surprising given the fact that we analyzed approximately five times more pancreatic tissue when compared with some extra-pancreatic tissues (Table 1). These observations indicate that while peripheral sites of PrP^{Sc} infection are evident in both models following i.c. inoculation, these pathways may not be as prominent in the pancreas as in other peripheral tissues. This provides some support for the notion that prion-induced alteration of pancreatic endocrine function is not localized to the islet cells themselves. In part, our results are supported by previous studies using both western blot analysis (Carp *et al.* 1994) as well as bioassay methodology (Carp *et al.* 1990, Kimberlin & Wilesmith 1994), which concluded that the pancreas was not a major target of early prion infection. However, low prion titers are present in pancreata from clinical 139H hamsters but are ~6000- to 10 000-fold lower than the titers in brain (Carp *et al.* 1990, Kimberlin & Wilesmith 1994, Ye *et al.* 1994a). Since it is not clear when PrP^{Sc} deposition occurs in the pancreas of the 139H scrapie-infected hamsters, we can not completely rule out the islet cells as the primary site of prion-induced alteration of pancreatic endocrine function. Clearly, researchers studying 139H scrapie-infected hamsters (using electron microscopy, Ye *et al.* 1997) and immunostaining procedures (Ye & Carp 1995) have identified PrP^{Sc} deposition within pancreata and have described prominent pathological changes specifically within the islet cells (Ye & Carp 1995, Ye *et al.* 1997) which are reminiscent of increased protein synthesis, enhanced degranulation, and necrosis (Ye *et al.* 1997). Hamsters receiving i.c. inoculation with a prion strain that does not cause obesity (i.e., 263K) do not develop alterations of pancreatic endocrine function/pathological changes and show no evidence of PrP^{Sc} deposition in the pancreas as evaluated by immunohistochemistry (Carp *et al.* 1994). On the other hand, PrP^{Sc} deposition has been documented in islet cells of rodents infected with other strains of prions, yet, alteration of pancreatic endocrine function does not always occur (Kim *et al.* 1987, Carp *et al.* 1990, McBride *et al.* 1992, Kimberlin & Wilesmith 1994, Ye *et al.* 1994a). Clearly, pancreatic islets contain mRNA for the normal prion protein (Atouf *et al.* 1994) and are, therefore, theoretically capable of replicating prion infection. However, no studies have established the temporal relationship between PrP^{Sc} infection within pancreatic islet cells, histopathological changes, and altered pancreatic endocrine function. In this regard, additional spatiotemporal studies are needed that identify prion-induced pathology within islet cells and evaluate islet cell function at the cellular and/or molecular level.

139H scrapie-infected hamsters appear to have altered leptin sensitivity whereas HY TME-infected hamsters have normal feed intake despite diminished leptin concentrations

Hyperphagia in spite of hyperleptinemia was observed in the 139H scrapie model, which could be the result of strain-specific disruption of the central neuroendocrine systems involved in feed intake regulation. This is suggested because the increased body weights in the 139H scrapie-infected

hamsters coincided with hyperphagia as well as with increases in non-fasting insulin and fasting levels of leptin. Such increases in both of these hormones are thought to strongly oppose such increases in feed intake and, ultimately, oppose uncontrolled weight gain if central hypothalamic nuclei are intact and respond appropriately to negative feedback (for reviews, see Woods & Seely 2000).

In non-fasted HY TME hamsters, diminished levels of leptin, which may be the result of chronic catabolism and an overall reduction in total adipose tissue mass, should ordinarily stimulate compensatory feed intake in hamsters (Schneider *et al.* 2000). However, feed intake was normal throughout the experimental period in HY TME-infected hamsters, even in those which had lost significant body weight. Therefore, in both prion disease models, the evidence indicates prion-induced alteration of the central regulation of food intake. In the case of 139H scrapie-infected hamsters, non-fasted levels of leptin were generally diminished, consistent with the observed hyperphagia. However, fasting-induced decreases in leptin (which was a response normally observed in mock hamsters) were gradually diminished in 139H scrapie-infected hamsters, resulting in increased levels of fasting leptin, which failed to inhibit feed intake. The data also indicate that the mechanisms regulating leptin secretion during fasting are altered by 139H scrapie infection. In the HY TME model, there is a lack of increased feed intake when low concentrations of serum leptin are evident and when body weight is being lost. Such catabolism normally induces compensatory feeding in the starving hamster (Borer *et al.* 1979, Rowland 1984) but failed to do so in HY TME-infected hamsters.

PrP^{Sc} deposition in the neuroendocrine and/or autonomic nervous system may underlie alterations in both pancreatic endocrine function and leptin responsiveness

Two intriguing possibilities arise with respect to explaining our observations of prion-induced alteration of pancreatic endocrine function and central regulation of leptin-mediated feed intake. One involves alteration of brain stem nuclei and autonomic outflow to the pancreas, whereas the other involves alteration of hypothalamic nuclei and neuroendocrine function. The possibility that PrP^{Sc} deposition within the hypothalamus causes the observed changes in pancreatic function has been suggested previously (Ye *et al.* 1994a,b, 1997, Ye & Carp 1995). A study employing stereotaxic injection of a prion strain (i.e. ME7) into the hypothalami of mice caused obesity and perturbations in glucose tolerance, while injection of the same strain into the cerebral cortex did not (Kim *et al.* 1987). However, as pointed out by Cunningham *et al.* (2005), the underlying relationship between PrP^{Sc} deposition and specific pathology (e.g., spongiform lesions, plaque formation, etc.) within tissues and the resulting effects on cellular function remains elusive. Nevertheless, prion-strain-specific targeting of the hypothalamus has been shown at clinical disease in both hamster

and mouse models of prion disease (DeArmond *et al.* 1993). In one study (Carp *et al.* 1984), scrapie strains that induced obesity in mice (e.g., ME7 and 22L) also caused intense vacuolation and lesions within the hypothalamus compared with strains that did not cause obesity (e.g., 22A). The observations we have made with respect to altered leptin sensitivity and dysregulation of feed intake, as well as the alterations of water intake, may also support the notion of prion-strain-specific targeting. In this regard, the lateral hypothalamic area, paraventricular, ventromedial, and arcuate nuclei appear to be immunopositive for PrP^{Sc} deposition at least at clinical disease in 139H scrapie-infected hamsters (Ye & Carp 1996, Ye *et al.* 1998, Sigurdson *et al.* 2001). Unfortunately, a temporal study evaluating strain-specific pathology within these areas has not been conducted. Nonetheless, based on classic ablation experiments, these sites are potentially involved in the anabolic syndrome in 139H scrapie-infected hamsters as well as the catabolic syndrome in HY TME-infected hamsters. This is suggested because, in rats, bilateral lesions to several of the listed hypothalamic nuclei induce obesity, hyperphagia and hyperinsulinemia (Bray *et al.* 1981), whereas a midline stereotaxic lesion that destroys the lamina terminalis and preoptic periventricular area produces hypodipsia, yet, feed intake is not compromised (Johnson & Buggy 1978). These studies describe the basic neuroanatomical loci that are the putative targets of prion-strain-specific lesions, which in turn, may underlie the strain-specific alterations in endocrine homeostasis that we have observed.

Since we cannot rule out the pancreas as the primary site of prion-induced alteration in pancreatic endocrine function, it seems appropriate to elaborate on the potential pathways that may be involved therein. Pancreatic islet cells are richly innervated by autonomic nerves (for review, see Ahren 2000) for which the terminals lie in close juxtaposition to the islet cells that secrete either insulin or glucagon (Ahren *et al.* 1986). Since autonomic disturbances (e.g., cardiovascular dynamics) have been described in other prion diseases (Pomfrett *et al.* 2004), it is plausible that alterations in parasympathetic or sympathetic outflow to the pancreas may underlie the prion-induced alteration of the pancreas that we have described. The specific pathways may include parasympathetic nerve fibers of postganglionic origin that emanate from intrapancreatic ganglia (Ahren *et al.* 1986, Brunicardi *et al.* 1995), which are controlled by preganglionic fibers originating almost exclusively in the dorsal motor nucleus of the vagus (DMNV; Ahren *et al.* 1986, Berthoud & Powley 1990, Brunicardi *et al.* 1995). Interestingly, the DMNV is an early target of PrP^{Sc} infection in hamsters receiving oral inoculation with the 139H scrapie agent (Beekes *et al.* 1998), as well as in deer with naturally occurring chronic wasting disease (Sigurdson *et al.* 2001) and in sheep with clinical scrapie (van Keulen *et al.* 2000, Ersdal *et al.* 2003). Several studies show that parasympathetic neurotransmitters are localized to islet parasympathetic nerves, are released directly by vagal stimulation and can induce insulin secretion (Ahren &

Taborsky 1986). Hence, it is plausible that prion-induced alteration of cell bodies within the DMNV could impact parasympathetic outflow to the pancreas in a manner that causes hypersecretion of insulin, such as in the 139H scrapie model. This may be further supported by our observations of glucose tolerance in 139H scrapie-infected hamsters, which suggest that pancreatic islets are non-responsive to elevated glucose and already maximally secreting insulin even during the fasted state.

Just as parasympathetic neurons mainly stimulate the secretion of insulin, sympathetic neurons mainly stimulate the secretion of glucagon (Ahren & Taborsky 1986, deJong *et al.* 1997). Many sympathetic neurons that directly innervate pancreatic islet cells originate as preganglionic cell bodies within the hypothalamus (Ahren & Taborsky 1986, deJong *et al.* 1997). Perhaps, the increased glucagon secretion observed in HY TME-infected hamsters could be the result of prion-strain-specific alteration of these particular hypothalamic areas in a manner that increases sympathetic outflow to the pancreas. A more detailed analysis of strain-specific PrP^{Sc} deposition within the hypothalamus may help elucidate the alterations in pancreatic endocrine function, as well as the marked alterations in water intake we observed. For example, if the sympathetic cell bodies that innervate the pancreas are located in an area of the hypothalamus, which is also known to control water intake in the hamster, the marked hypodipsia that was observed in HY TME-infected hamsters may also be explained by prion-induced pathology at these sites.

In conclusion, the hypothalamus and the brain stem contain neurons that are intimately involved in the control of energy homeostasis and are capable of regulating autonomic outflow to the pancreas and hence secretion of insulin and glucagon. Both sites appear to be targets of PrP^{Sc} deposition in a prion-strain-dependent manner. Therefore, it is feasible that an interaction between strain-specific central targeting of 139H scrapie and HY TME and prion-induced pathology within distinct neuroendocrine and/or autonomic pathways could be responsible for the preclinical, strain-specific alterations in energy homeostasis that we observed.

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References

- Ahren B 2000 Autonomic regulation of islet hormone secretion-implications for health and disease. *Diabetologia* **43** 393–410.
- Ahren B & Taborsky GJ, Jr 1986 The mechanism of vagal nerve stimulation of glucagon and insulin secretion in the dog. *Endocrinology* **118** 1551–1557.
- Ahren B, Taborsky GJ, Jr & Porte D, Jr 1986 Neuropeptidergic versus cholinergic and adrenergic regulation of islet hormone secretion. *Diabetologia* **29** 827–836.
- Atouf F, Scharfmann R, Lasmez C & Czernichow P 1994 Tight hormonal control of PrP gene expression in endocrine pancreatic cells. *Biochemical and Biophysical Research Communications* **201** 1220–1226.
- Bartz JC, Bessen RA, McKenzie D, Marsh RF & Aiken JM 2000 Adaptation and selection of prion protein strain conformations following interspecies transmission of transmissible mink encephalopathy. *Journal of Virology* **74** 5542–5547.
- Bartz JC, Kincaid AE & Bessen RA 2003 Rapid prion neuroinvasion following tongue infection. *Journal of Virology* **77** 583–591.
- Bartz JC, Dejoia C, Tucker T, Kincaid AE & Bessen RA 2005 Extraneural prion neuroinvasion without lymphoreticular system infection. *Journal of Virology* **79** 11858–11863.
- Beekes M, McBride PA & Baldauf E 1998 Cerebral targeting indicates vagal spread of infection in hamsters fed with scrapie. *Journal of General Virology* **79** 601–607.
- Berthoud HR & Powley TL 1990 Identification of vagal preganglionics that mediate cephalic phase insulin response. *American Journal of Physiology* **258** R523–R530.
- Bessen RA & Marsh RF 1992 Identification of two biologically distinct strains of transmissible mink encephalopathy in hamsters. *Journal of General Virology* **73** (Pt2) 329–334.
- Borer KT, Rowland N, Mirow A, Borer RC Jr & Kelch RP 1979 Physiological and behavioral responses to starvation in the golden hamster. *American Journal of Physiology* **236** E105–E112.
- Bray GA, Inoue S & Nishizawa Y 1981 Hypothalamic obesity: the autonomic hypothesis and the lateral hypothalamus. *Diabetologia* **20** 366–377.
- Brunnicardi FC, Shavelle DM & Andersen DK 1995 Neural regulation of the endocrine pancreas. *International Journal of Pancreatology* **18** 177–195.
- Busiguina S, Fernandez AM, Barrios V, Clark R, Tolbert DL, Berciano J & Torres-Aleman I 2000 Neurodegeneration is associated to changes in serum insulin-like growth factors. *Neurobiological Disorders* **7** 657–665.
- Capucchio MT, Guarda F, Pozzato N, Coppolino S, Caracappa S & DiMarco V 2001 Clinical signs and diagnosis of scrapie in Italy: a comparative study in sheep and goats. *Journal of Veterinary Medicine* **48** 23–31.
- Carp RI, Callahan SM, Sersen EA & Moretz RC 1984 Preclinical changes in weight of scrapie-infected mice as a function of scrapie agent-mouse strain combination. *Intervirology* **21** 61–69.
- Carp RI, Kim YS & Callahan SM 1989 Scrapie-induced alterations in glucose tolerance in mice. *Journal of General Virology* **70** 827–835.
- Carp RI, Kim YS & Callahan SM 1990 Pancreatic lesions and hypoglycemia-hyperinsulinemia in scrapie-injected hamsters. *Journal of Infectious Diseases* **161** 462–466.
- Carp RI, Callahan SM, Yu Y & Sersen E 1993 Analysis of host genetic control of scrapie-induced obesity. *Archives of Virology* **133** 1–9.
- Carp RI, Ye X, Kascak RJ & Rubenstein R 1994 The nature of the scrapie agent. Biological characteristics of scrapie in different scrapie strain-host combinations. *Annals of the New York Academy of Sciences* **724** 221–234.
- Collinge J 2001 Prion diseases of humans and animals: their causes and molecular basis. *Annual Review of Neuroscience* **24** 519–550.
- Cunningham C, Deacon RM, Chan K, Boche D, Rawlins JN & Perry VH 2005 Neuropathologically distinct prion strains give rise to similar temporal profiles of behavioral deficits. *Neurobiological Disorders* **18** 258–269.

- DeArmond SJ, Yang SL, Lee A, Bowler R, Taraboulos A, Groth D & Prusiner SB 1993 Three scrapie prion isolates exhibit different accumulation patterns of the prion protein scrapie isoform. *PNAS* **90** 6449–6453.
- Ersdal C, Ulvund MJ, Benestad SL & Tranulis MA 2003 Accumulation of pathogenic prion protein (PrP^{Sc}) in nervous and lymphoid tissues of sheep with subclinical scrapie. *Veterinary Pathology* **40** 164–174.
- Gayraud V, Picard-Hagen N, Grino M, Sauze N, Grandjean C, Galea J, Andreoletti O, Schelcher F & Toutain PL 2000 Major hypercorticism is an endocrine feature of ewes with naturally occurring scrapie. *Endocrinology* **141** 988–994.
- Hecker R, Taraboulos A, Scott M, Pan KM, Yang SL, Torchia M, Jendroska K, DeArmond SJ & Prusiner SB 1992 Replication of distinct scrapie prion isolates is region specific in brains of transgenic mice and hamsters. *Genes and Development* **6** 1213–1228.
- Heikenwalder M, Julius C & Aguzzi A 2007 Prions and peripheral nerves: a deadly rendezvous. *Journal of Neuroscience Research* **85** 2714–2725.
- Herzog C, Riviere J, Lescoutra-Etcheagaray N, Charbonnier A, Leblanc V, Sales N, Deslys JP & Lasmezas CI 2005 PrPTSE distribution in a primate model of variant, sporadic, and iatrogenic Creutzfeldt–Jakob disease. *Journal of Virology* **79** 14339–14345.
- Johnson AK & Buggy J 1978 Periventricular preoptic-hypothalamus is vital for thirst and normal water economy. *American Journal of Physiology* **234** R122–R129.
- de Jong A, Strubbe JH & Steffens AB 1977 Hypothalamic influence on insulin and glucagon release in the rat. *American Journal of Physiology* **233** E380–E388.
- Kascak RJ, Rubenstein R, Merz PA, Tonna-DeMasi R, Fersko R, Carp RI, Wisniewski HM & Diringier H 1987 Mouse polyclonal and monoclonal antibody to scrapie-associated fibril proteins. *Journal of Virology* **61** 3688–3693.
- van Keulen LJ, Schreuder BE, Vromans ME, Langeveld JP & Smits MA 2000 Pathogenesis of natural scrapie in sheep. *Archives of Virology* **16** 57–71.
- Kim YS, Carp RI, Callahan SM & Wisniewski HM 1987 Scrapie-induced obesity in mice. *Journal of Infectious Diseases* **156** 402–405.
- Kim YS, Carp RI, Callahan SM & Wisniewski HM 1988 Scrapie-induced obesity. *Proceedings of the Society of Experimental and Biological Medicine* **189** 21–27.
- Kimberlin RH, Walker CA & Fraser H 1989 The genomic identity of different strains of mouse scrapie is expressed in hamsters and preserved on reisolation in mice. *Journal of General Virology* **70** 2017–2025.
- Kimberlin RH & Wilesmith JW 1994 Bovine spongiform encephalopathy. Epidemiology, low dose exposure and risks. *Annals of the New York Academy of Sciences* **724** 210–220.
- McBride PA, Eikelenboom P, Kraal G, Fraser H & Bruce ME 1992 PrP protein is associated with follicular dendritic cells of spleens and lymph nodes in uninfected and scrapie-infected mice. *Journal of Pathology* **168** 413–418.
- Miller MW & Williams ES 2002 Detection of PrP(CWD) in mule deer by immunohistochemistry of lymphoid tissues. *Veterinary Record* **151** 610–612.
- Pomfrett CJ, Glover DG, Bollen BG & Pollard BJ 2004 Perturbation of heart rate variability in cattle fed BSE-infected material. *Veterinary Record* **154** 687–691.
- Prusiner SB 1987 Prions and neurodegenerative diseases. *New England Journal of Medicine* **317** 1571–1581.
- Prusiner SB 1998 Prions. *PNAS* **95** 13363–13383.
- Rowland N 1984 Metabolic fuel homeostasis in golden hamsters: effects of fasting, refeeding, glucose, and insulin. *American Journal of Physiology* **247** R57–R62.
- Salman MD 2003 Chronic wasting disease in deer and elk: scientific facts and findings. *Journal of Veterinary Medical Science* **65** 761–768.
- Schelcher F, Picard-Hagen N, Laroute V, Gayraud V, Popot MA, Andreoletti O & Toutain PL 1999 Corticoid concentrations are increased in the plasma and urine of ewes with naturally occurring scrapie. *Endocrinology* **140** 2422–2425.
- Schneider JE, Blum RM & Wade GN 2000 Metabolic control of food intake and estrous cycles in syrian hamsters. I. Plasma insulin and leptin. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **78** R476–R485.
- Sigurdson CJ, Spraker TR, Miller MW, Oesch B & Hoover EA 2001 PrPCWD in the myenteric plexus, vagosympathetic trunk and endocrine glands of deer with chronic wasting disease. *Journal of General Virology* **82** 2327–2334.
- Srinivasappa J, Asher DM, Pomeroy KL, Murphy LJ, Wolff AV, Yoon JW, Gajdusek DC & Notkins AL 1989 Scrapie-induced diabetes mellitus in hamsters. *Microbial Pathogenesis* **7** 189–194.
- Viguie C, Chilliard Y, Gayraud V, Picard-Hagen N, Monget P, Dutour A & Toutain PL 2004 Alterations of somatotrophic function in prion disease in sheep. *Journal of Endocrinology* **183** 427–435.
- Woods SC & Seeley RJ 2000 Adiposity signals and the control of energy homeostasis. *Nutrition* **16** 894–902.
- Ye X & Carp RI 1995 The pathological changes in peripheral organs of scrapie-infected animals. *Histology and Histopathology* **10** 995–1021.
- Ye X & Carp RI 1996 Histopathological changes in the pituitary glands of female hamsters infected with the 139H strain of scrapie. *Journal of Comparative Pathology* **114** 291–304.
- Ye X, Carp RI & Kascak RJ 1994a Histopathological changes in the islets of Langerhans in scrapie 139H-affected hamsters. *Journal of Comparative Pathology* **110** 153–167.
- Ye X, Carp RI, Yu Y, Kozielski R & Kozlowski P 1994b Hyperplasia and hypertrophy of B cells in the islets of Langerhans in hamsters infected with the 139H strain of scrapie. *Journal of Comparative Pathology* **110** 169–183.
- Ye X, Scallet AC & Carp RI 1997 The 139H scrapie agent produces hypothalamic neurotoxicity and pancreatic islet histopathology: electron microscopic studies. *Neurotoxicology* **18** 533–545.
- Ye X, Scallet AC, Kascak RJ & Carp RI 1998 Astrocytosis and amyloid deposition in scrapie-infected hamsters. *Brain Research* **809** 277–287.

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