THE AGING PROCESS DECONSTRUCTED: GLUCOSE PRODUCTION, CANCER RESISTANCE AND LONGEVITY

Jennifer McKee Alderman

"A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in the Department of Nutrition, School of Medicine, Gillings School of Global Public Health (Nutritional Biochemistry)."

Chapel Hill
2009

Approved by:

Dr. Melinda Beck

Dr. David Clemmons

Dr. Rosalind Coleman

Dr. Terry Combs

ABSTRACT

JENNIFER MCKEE ALDERMAN: The Aging Process Deconstructed:
Glucose Production, Cancer Resistance and Longevity
(Under the direction of Terry P. Combs)

The aging process affects all mammals and is typically seen with a gradual decline in overall system functionality. This can affect organ and immune function, resulting in increased susceptibility to diseases such as cancer. There are many theories as to specific mechanisms of longevity; we investigate the neuroendocrine regulation of glucose utilization as a potential mediator. Animal models are valuable tools in our efforts to perform gerontological research. Increased lifespan in rodents and mice has been observed through calorie restriction and single mutations, such as $Pit^{-/-}$ and $Proph1^{-/-}$. Our results in *Snell* dwarf mice suggest that the pituitary gland and adipose tissue are part of a neuroendocrine loop that lower the risk of cancer during aging by reducing the availability of glucose.

To my Mom,
Carolyn Sue Whitson McKee (1947-1994),
whose love will never be forgotten.

To my Dad,
Dr. Thomas E. McKee,
whose indefatigable courage, wisdom, and love have made him my hero.

ACKNOWLEDGEMENTS

I am indebted to many people, both singularly and grouped. To undertake this degree with my unorthodox lifestyle has required tremendous support and encouragement. First, I would like to thank my husband, Eric, for his love and unwavering belief that I would be successful in this endeavor. I admire his exemplary courage and dedication serving our nation and protecting its citizens; I am incredibly proud of him. Second, my family: Misty, Dad, Brandon, Linda, John, Gloria, and Margaret. They have provided me with love, support, sympathetic ears, and invaluable assistance during my stressful moments and Eric's deployments, for which I am eternally grateful. Lastly, for comedic relief, I am thankful to my crazy dogs, Ludo and Sweetpea, whose infamous exploits and ridiculous shenanigans kept me simultaneously laughing, exasperated, and sane on a daily basis.

I would like to express gratitude to my committee: Drs Beck, Clemmons, Coleman, and Combs. Thank you for your masterful expertise and diligence; I am honored.

I am also thankful to the directors of the UNC-Duke Michael Hooker Proteomics Center, Drs Carol Parker and Maria Warren. Their willingness to help a newbie participate in the complicated realm of mass spec and share their super software is greatly appreciated.

I would also like to acknowledge co-authors of previously published material and my colleagues who contributed ideas, words, and shared data: Natasha L. Brooks, Sneha B. Naik, Jonathan M. Gutierrez, Urmila Srinivas, Kristen B. Ziara, Linhong Jing, Xian Chen Dr. Flurkey, Dr. Boysen, Dr. Bronson, Dr. Klebanov, Dr. Swenberg, Dr. Stridsberg, Dr. Harrison, Dr. Helle, Valerie Kohdush, Kajal Patel, Essraa Bayoumi, Heather Paich, Michael DePetrillo, Angie Gluesenkamp, Antonia Hartley, Veronica Verhoff, Katherine Zhavodni, Elizabeth Alderman, Carl Raetzsch, Pete Hosick, Rachel Bryan, Erin Thompson, and Melissa Asmar.

Lastly, I am deeply grateful and indebted to my professor, Dr. Terry P. Combs. I could not have survived without his steady support, kind understanding, quiet humor and good grace. I am privileged to consider him both a mentor and my friend. Thank you.

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ACTH: adrenalcorticotropic hormone

AMPK: AMP activated protein kinase

ATP: adenosine triphosphate

CGA: chromogranin A

CGB: chromogranin B

CR: calorie restriction

DW: dwarf

MEK-ERK: mitogen activated protein kinase- extracellular signal related kinase

$FADH_2$: flavin adenine dinucleotide (reduced)

FSH: follicle stimulating hormone

G6PASE: glucose-6-phosphatase

GH: growth hormone

GLUT4: glucose transporter 4

IGF-1: insulin-like growth factor 1

LH: luteinizing hormone

MDA: malondialdehyde

mtDNA: mitochondrial DNA

NADH: nicotiamide adenine dinucleotide (reduced)

nDNA: nuclear DNA

OH8dG: 8-hydroxy-2-deoxyguanosine

PEPCK: phosphoenolypyruvate carboxykinase

PRL: prolactin

SEM: standard error of the mean

SGI: secretogranin I

SGII: secretogranin II

TSH: thyroid stimulating hormone

WT: wild-type

CHAPTER I

EXPERIMENT: CANCER RESISTANCE IN *SNELL* DWARF MICE

ABSTRACT

Pit1 null (*Snell* dwarf) mice are long-lived mouse models that are resistant to cancer. Endogenous glucose production is lower in *Snell* dwarf than control mice during fasting. Does the reduction of glucose production provide resistance to cancer in *Snell* dwarf mice? First, we examined whether endogenous glucose production is also lower in *Snell* dwarf mice during feeding. The reduction of endogenous glucose production during feeding and fasting could reduce glucose utilization by cancer cells and provide resistance to cancer. Inhibition of endogenous glucose production by injection of glucose was enhanced in 13-month-old female *Snell* dwarf mice. Second, we compared the incidence of cancer at time of death in old *Snell* dwarf and control mice. Only 18% of old *Snell* dwarf mice had malignant lesions at the time of death compared to 82% of control mice. The median ages at death for *Snell* dwarf and control mice in this study were 33 and 26 months, respectively. Elevated circulating adiponectin, a hormone produced by adipose tissue, was observed in 13 month old female *Snell* dwarf mice. The elevation of adiponectin may provide *Snell* dwarf mice with resistance to cancer by inhibiting endogenous glucose production. Like *Snell* dwarf mice, Proph1 null (*Ames* dwarf) mice are long-lived mouse models that lack GH, PRL and TSH and show reduced IGF-I and elevated adiponectin. However, in contrast to *Snell* dwarf mice, old *Ames* dwarf mice show a high incidence of cancer at the time of death similar to control mice. Hence, endocrine factors other than elevated adiponectin, reduced IGF-I and a lack of GH, PRL and TSH provide old *Snell* dwarf mice with cancer resistance. Proteomics analysis of pituitary secretions revealed the lack of GH and PRL, the secretion of ACTH and elevated secretion of Chromogranin B and Secretogranin II in *Snell* dwarfs. We confirmed the elevation of circulating Chromogranin B and Secretogranin II in *Snell* dwarf mice by radioimmunoassay. In summary, these results suggest that the pituitary gland and adipose tissue are part of a complex neuroendocrine system that inhibits endogenous glucose production and thereby reduces the risk of cancer.

INTRODUCTION

Snell dwarf mice are valuable laboratory models for investigating the mechanisms of cancer and aging. *Snell* dwarf mice are homozygous for the $Pit1^{dw}$ allele which leads to the loss of the transcription factor Pit1 (Li et al., 1990). *Snell* dwarf mice are deficient in growth hormone (GH), prolactin (PRL) and thyroid stimulating hormone (TSH) due to hypoplasia of cells that produce these hormones in the anterior pituitary. *Snell* dwarf mice are resistant to chemically induced cancer and live longer than control mice which are either homozygous or heterozygous for the wild-type Pit1 allele (Bielschowsky and Bielschowsky, 1959; Flurkey et al., 2001; Rennels et al., 1965).

Endogenous glucose production and whole-body glucose utilization are lower in *Snell* dwarf mice than control mice during fasting (Brooks et al., 2007c). Cancer cells depend on high levels of anaerobic glycolysis rather than oxidative phosphorylation for ATP production (Brown, 1999; Gullino et al., 1967; Matoba et al., 2006). Glucose deprivation causes cancer cells *in vitro* to undergo cell death more readily than healthy cells (Elstrom et al., 2004b; Funes et al., 2007; Shim et al., 1998). Elevated circulating glucose increases the risk of cancer and diminishes the effectiveness of medical treatment in cancer patients (Caudle et al., 2008; Malin et al., 2005; Stattin et al., 2007). Hence, reduced endogenous glucose production may provide resistance to cancer in *Snell* dwarf mice by depriving cancer cells of energy.

Glucose homeostasis differs dramatically between the fed and fasted states. During feeding (a) the concentration of exogenous glucose in the circulation from the gastrointestinal tract is elevated, (b) the concentrations of total circulating glucose and insulin are elevated, (c) circulating glucose is used for energy and storage and (d) endogenous glucose production is not needed to replenish the disappearance of circulating glucose. Endogenous glucose production was previously measured in *Snell* dwarf mice during fasting (Brooks et al., 2007c). Therefore, the first goal of this study was to determine whether elevated inhibition of endogenous glucose production in *Snell* dwarf mice extends to the fed state.

Hypophysectomy, surgical removal of the pituitary gland, was used as endocrine therapy before highly specific chemicals and monoclonal antibodies were available to reduce hormone action to block hormone binding (Luft et al., 1955). Among pituitary hormones, the lack of GH combined with low levels of IGF-I, a hormone stimulated mainly by GH, plays a key role in cancer resistance. GH deficient rats are highly resistant to chemical induction of cancer unless GH is provided by injection (Shen et al., 2007). Furthermore, the suppression of IGF-I could provide resistance to cancer by reducing whole-body glucose utilization (Rossetti et al., 1991).

Resistance to cancer in *Snell* dwarf mice could also be mediated by the elevation of circulating adiponectin (Brooks et al., 2007c; Combs et al., 2003). Adiponectin, a hormone produced exclusively by adipocytes, the lipid storing cells in fat tissue, inhibits endogenous glucose production (Brooks et al., 2007c; Combs et al., 2001; Combs et al., 2004). Numerous clinical studies show that cancer risk is associated with

elevated circulating adiponectin (Chen et al., 2006; Korner et al., 2007; Mantzoros et al., 2004; Miyoshi et al., 2003; Tworoger et al., 2007).

Elevated adiponectin has been reported in several GH deficient and long-lived mouse models (Berryman et al., 2004; Combs et al., 2003; Wang et al., 2006). Adiponectin inhibits endogenous glucose production and stimulates fatty acid utilization through its intracellular target, AMP-activated protein kinase (Brooks et al., 2007a; Nawrocki et al., 2006; Tomas et al., 2002; Yamauchi et al., 2002). Adiponectin inhibits endogenous glucose production in the liver by suppressing G6Pase and PEPCK, rate limiting enzymes in hepatic glucose production (Combs et al., 2004). Gene expression array data in GH deficient mice such as the *Snell* dwarf, *Ames* dwarf, GH release hormone receptor-null (*little*) and GH receptor-null mice, support the conclusion that fatty acid oxidation is elevated in these long-lived and cancer resistant mouse models (Stauber et al., 2005). Exercise training in humans enhances fatty acid metabolism through AMPK (Winder, 2001).

Ames dwarf mice are homozygous for the Prop1^{df} allele which leads to the loss of the transcription factor Prop1 (Sornson et al., 1996). Prop1 regulates the expression of Pit1 and the development of thyrotrophs, somatotrophs and lactotrophs in the anterior pituitary from Rathke's pouch. As a result, *Ames* dwarf mice are deficient in GH, PRL and TSH similar to *Snell* dwarf mice. *Ames* dwarf mice live longer than control mice which are either homozygous or heterozygous for the wild-type Prop1 allele (Brown-Borg et al., 1996; Ikeno et al., 2003). The median age of death for *Ames* dwarf and control mice was 36 and 26 months, respectively; however, despite having a similar endocrine profile as *Snell* dwarf mice, old *Ames* dwarf mice exhibit a high incidence of cancer at the time of death (Ikeno et al., 2003). Necropsy at the time of death revealed malignant lesions in 72% of *Ames* dwarf mice compared to 95% in control mice. Although young *Snell* dwarf mice exhibit resistance to chemically induced tumors, data on naturally occurring murine cancers in old *Snell* dwarf mice is currently not available. Thus, our second goal was to determine whether old *Snell* dwarf mice also have a high incidence of cancer at the time of death as *Ames* dwarf mice.

After discovering greater inhibition of endogenous glucose production by intravenous injection of glucose (aim 1) and resistance to cancer at the time of death in old *Snell* dwarf mice (aim 2), we examined by proteomics analysis whether the pituitary of *Snell* dwarf mice overproduces other hormones or lacks other hormones besides GH, PRL and TSH that may play a role in the resistance to cancer (aim 3). Previous proteomics analyses of whole anterior pituitaries from outbred mice verified production of all known pituitary hormones except for TSH (Blake et al., 2005). Therefore, our third and final goal was to compare the proteins secreted from the pituitaries of *Snell* dwarf and control mice.

METHODS

Mice. Unless indicated otherwise 12-14 month old female mice in the F1 (DWxB6) background were used in the present study. Mice were housed in ventilated isolator cage systems in a pathogen-free isolator barrier facility at 23^{o}C, 55% humidity on a 12-hour light/12-hour dark cycle. Mice received a standard chow diet consisting 73% carbohydrate, 18% protein, 4% fat, 5% ash (Purina). Mice were sacrificed by cervical dislocation for experiments requiring dissection of tissues. Experimental procedures were approved by the Institutional Animal Care and Use Committees at the University of North Carolina.

Intravenous Glucose Tolerance Test. Mice were fasted after 7 am and studied between 1 and 3 pm. In the afternoon, mice received a single intra-venous injection of glucose (0.8 mg/g bodyweight) mixed with tritium labeled glucose, D-[3-^{3}H]glucose (^{3}H label on carbon 3 glucose) from Amersham Biosciences (Beard et al., 1986). The mixture was injected directly into the circulation of conscious, free-moving mice through the ophthalmic plexus. Blood samples (10-20 μL) were collected from the tail tip 2.5, 5, 10, 20 and 30 minutes after injection for the measurement of glucose and radioactivity as previously described (Brooks et al., 2007c). The tail was nicked 2-4 mm from the tip always below the end of the vertebrae. Blood was collected from the tail-vein of free moving mice. The fraction of circulating glucose representing the injected glucose was calculated from the specific activity of the [^{3}H]glucose-glucose solution injected (300 cpm per μg glucose). HPLC analysis was performed to verify that the radioactivity measured in dried plasma by scintillation counter represented [^{3}H]glucose. An Agilent 1100 HPLC system connected to a beta-RAM Model 2B Detector (IN/US Systems) was used. An Aminex HPX-87C polystyrene divinylbenzene resin column (Bio-Rad) was used at 80^{o}C using a 0.6 mL/min flow of water. Mutant and control mice were always paired for intravenous glucose tolerance test.

Cancer Evaluation at Necropsy. Gross pathologic examination was performed while gathering life span data at the Jackson Laboratories. Necropsy was performed on female *Snell* dwarf mice and control mice from a cohort of F1 (DWxC3He) mice. Cages were checked daily and necropsy was performed immediately when a dead mouse was found. The median age at time of death was 33 months for *Snell* dwarf mice and 26 months for control mice. A total of 11 *Snell* dwarf and 38 control mice underwent necropsy. Histology was used to determine whether neoplastic lesions were benign or malignant. The neoplastic lesions identified by histology included histiocytic sarcoma, lymphoma, mammary adenocarcinoma, fibrosarcoma, hepatocellular carcinoma, leiomyosarcoma, myelogenous leukemia and hemangiosarcoma.

Proteomics Analysis of Pituitary Secretions. Anterior pituitary glands from 12-14 month old *Snell* dwarf and age-matched control mice were placed in serum-free modified Eagle's media immediately after dissection and were incubated at 37^{o} C for 1 hour. The media was transferred to new tubes and centrifuged at 45,000 RPM for 1 hour. Proteomics analyses were performed at the UNC-Duke Proteomics Center. The supernatant was digested with trypsin at 37^{o} C for 1 hour and was analyzed by nano LC/MS/MS

using a Waters API-US Q-Tof, equipped with a Waters capLC system and a 75µ id x 15 cm PepMap C18 column (Dionex). The supernatants were analyzed by nanoLC/MS/MS on a ThermoFisher Orbitrap hybrid FT-MS, equipped with an Eksigent nanoLC system, New Objective nanospray source and a nanobore PicoFrit column (ProteoPep^{TM} II C18, 50µm id x 10.0 cm, 10 µm tip size, New Objective). The Orbitrap data was processed using ThermoFisher's Bioworks 3.3.1 software, the Sequest search engine and the FASTA Protein Database. A comprehensive report of the protein functions was prepared using BLAST on the NCBInr database. Protein expression ratios were determined with GE Healthcare's DecyderMS software.

PCR Reactions and Northern Blot Analysis-Genotyping of Pit1^{dw} allele carriers for breeding. The Pit1^{dw} allele was detected from tail DNA to genotype breeding pairs by PCR using AGCTGCTAAGGATGCTCTGG (forward primer), CGTTTTTCTCTCTGCCTTCG (reverse primer) and PlatTAQ from Invitrogen on a BioRad thermocycler. These primers detected the Pit1^{dw} allele in breeding pairs (Szeto et al., 1996).

Reverse Transcriptase PCR. Mice were sacrificed by cervical dislocation between 1-3 pm. Tissues were frozen in liquid nitrogen immediately after dissection and stored at –70°C.RNA was isolated using a Qiagen kit according to the manufacturer 's protocol. cDNA was prepared with reverse transcriptase (Invitrogen) and random hexamers. The PCR primer pair sequences for Chromogranin A were AAGAGGCCTGAGTGCCCAAC and ACGCTCCTCCTCCTCTTCC (reverse). The PCR primer pair sequences for Chromogranin B were GACCAGGACCAGAGCCAG and GACCAGGACCAGAGCCAG while the primer pair sequences for Secretogranin II were GCAGTGGGAGGTCACAGAG and ATACCCACCCTTGGAGAGC (reverse). PCR conditions were 94^{o}, 55^{o} and 72^{o} C (35 cycles).

Northern Blot Analysis. Yield and purity of RNA were determined by spectrophotometric absorption analysis at 260/280 nm. Total RNA (5 µg) was electrophoresed on 1.2% agarose gel /6% formaldehyde and transferred to Hybond-XL membrane (Amersham). RNA was cross-linked to the membrane by ultraviolet irradiation and incubated at 65°C in hybridization buffer (GE Healthcare) overnight. Radiolabeled cDNA was introduced to the buffer, and the membranes were Blots were washed with 3xSSC, exposed to a PhosphorImager (Molecular Dynamics) screen for 24 h, and analyzed on a PhosphorImager using ImageQuant Software.

mRNA Probe synthesis. Radiolabeled probes were prepared by PCR using 2.5 mM [^{32}P]dCTP (Amersham). The Secretogranin II probe was prepared using TGCTGAAACGGCCCGAGC and CAGGCGTGTCCACTGGGAA. The Chromogranin B probe was prepared using TGCTGAAACGGCCCGAGC and CAGGCGTGTCCACTGGAA. An oligonucleotide (5 - C T T C C T C TA G ATA G TCAAGTTCGACCGTCT-3) specific for 18S rRNA (GenBank X01117) was end labeled with [^{32}P] d ATP using T4 polynucleotide kinase (Invitrogen) and used to confirm equal RNA loading.

Hormone Measurements. Adiponectin was measured in plasma from control and *Snell* dwarf mice by western blot analysis as previously described (Brooks et al., 2007c). Adiponectin was detected using rabbit antisera produced by the peptide antigen EDDVTTTEELAPALV representing murine adiponectin (amino acid residues 18-32) as previously described. Chromogranin A was measured using an antibody to human Chromogranin A amino acid sequence 324-337 coding for the peptide sequence ELEQEEERLSKEWE, which detects the WE-14 region of Chromogranin A. Chromogranin B was measured using an antibody to human Chromogranin B sequence 312-331 coding for the peptide sequence SEESNVSMASLGEKRDHHST (Stridsberg et al., 1995) Secretogranin II was measured as described previously using an antibody against the human Secretogranin II amino acid sequence 154–165 coding for the peptide QQWPERKLKHM. The detection limits were 10 fmol and the variability of replicate measurements was less than 10% (Nicol et al., 2002; Willis et al., 2008).

RESULTS

Glucose metabolism in *Snell* dwarf mice. Low endogenous glucose production and whole-body glucose utilization under fasting conditions were previously reported in *Snell* dwarf mice (Brooks et al., 2007c). Rather than the fasted state, the data presented ahead were obtained under conditions that mimic the fed state (Fig. 1). Control and *Snell* dwarf mice received a single intravenous injection of glucose and trace amounts of [^{3}H]glucose (0.8 mg/g body weight). Total plasma glucose was lower in *Snell* dwarfs at 2.5, 5, 10, 20 and 30 minutes after glucose injection and was found to be lower at all time points except baseline. Endogenous glucose production was lower in *Snell* dwarf mice at 2.5, 5, 10 and 20 minutes after glucose injection. The reduction of endogenous glucose production may restrict glucose utilization by cancer cells in *Snell* dwarf mice and thereby prevent cancer.

Incidence of cancer at time of death in old *Snell* dwarf mice. Low endogenous glucose production during feeding and fasting may deprive cancer cells of glucose thereby making *Snell* dwarf mice resistant to cancer. *Snell* dwarf mice are resistant to chemically induced cancers; however, the natural incidence of cancer in old *Snell* dwarf mice at the time of death has not been previously described (Bielschowsky and Bielschowsky, 1959; Flurkey et al., 2001; Rennels et al., 1965). Consistent with previous reports, significantly fewer *Snell* dwarf mice had neoplastic lesions at necropsy. Only 18% of the *Snell* dwarfs had at least one malignant lesion compared to 82% of the control mice (Fig. 2). These results contrast sharply with the evidence of high tumor incidence at time of death in old *Ames* dwarf mice (Ikeno et al., 2003).

Adiponectin levels in *Snell* dwarf mice. Adiponectin is a hormone that inhibits endogenous glucose production (Brooks et al., 2007c; Combs et al., 2001; Combs et al., 2004). If elevated adiponectin protects *Snell* dwarf mice from cancer, it is expected that it would remain elevated in older mice. The elevation of plasma adiponectin was previously reported in young *Snell* dwarf and *Ames* dwarf mice (Combs

et al., 2003; Wang et al., 2006). We found that plasma adiponectin was elevated in 12-14 month old *Snell* dwarf mice indicating a possible role for adiponectin in cancer resistance (Fig. 3).

Proteomics analysis of *Snell* dwarf pituitary secretions. The elevation of adiponectin and the reduction of GH may contribute to the reduction of glucose production and glucose utilization in *Snell* dwarf mice. However, to our knowledge, *Ames* dwarf mice show a similar endocrine profile as *Snell* dwarf mice yet *Ames* dwarfs are significantly less resistant to cancer than *Snell* dwarfs (Ikeno et al., 2003; Wang et al., 2006). Therefore, proteomics analysis was applied to determine whether any novel endocrine factors differ between *Snell* dwarf and control mice. Pituitary secretions were collected from *Snell* dwarf and control mice *ex vivo*. Proteomics analysis confirmed that the pituitary in the *Snell* dwarf produced ACTH but not GH or PRL. In addition, proteomics analysis revealed that *Snell* dwarf pituitary produced Chromogranin A, Chromogranin B and Secretogranin II (Table 1). The method identified a total of 107 proteins from control mouse pituitary and 52 proteins from *Snell* dwarf pituitary including proteins classified as nuclear, metabolic, protein structure, cell structure, signaling and secreted proteins. Detection of cellular proteins among pituitary secretions was indicative of *ex vivo* pituitary cell lysis. Decyder MS software analysis indicated that Chromogranin A, Chromogranin B and Secretogranin II were elevated based on matching peptide masses and retention time. PCR reactions were performed for Chromogranin A, Chromogranin B and Secretogranin II using anterior pituitary cDNA from control and *Snell* dwarf mice. Reverse transcriptase/PCR results showed that Chromogranin B and Secretogranin II mRNA are expressed in control as well as *Snell* dwarf pituitaries (data not shown). Primer pair sequences are provided in Materials and Methods. These results show confirm that Chromogranin B and Secretogranin II are expressed by the pituitary (Grino et al., 1989; Nicol et al., 2002; Wei et al., 1995).

Elevated circulating Chromogranin B and Secretogranin II levels were detected in *Snell* dwarf mice at $p<0.05$ by nonparametric Student t-test (Fig. 4A). The elevation of Chromogranin B and Secretogranin II led us to investigate whether the pituitary is a major determinant of circulating Chromogranin B and Secretogranin II levels by Northern blot analysis (Fig. 4B). Northern blot analysis of mRNA expression suggests that the pituitary and the adrenal glands are the major determinants of circulating Chromogranin B. On the other hand, the pituitary is not the main producer of Secretogranin II.

TABLE 1. Proteomics analysis of secreted proteins from the pituitary of *Snell* dwarf and control mice. Proteomics analysis confirmed that the pituitary of the *Snell* dwarf does not secrete GH or PRL and verified the secretion of ACTH, Chromogranin A, Chromogranin B and Secretogranin II. LH, FSH and TSH secretion was not detected from *Snell* dwarf or control and pituitaries. The plus sign (+) indicates detected, two plus signs (++) indicate a 2-fold difference and ND indicates not detected. Pituitary secretion levels were compared using quantitative proteomics analysis software (Decyder MS).

Secreted Pituitary Proteins by Proteomics Analysis	control	Snell dwarf
Growth Hormone	+	ND
Prolactin	+	ND
ACTH	+	+
Chromogranin A	+	++
Chromogranin B	+	++
Secretogranin II	+	++

FIGURE 1. Endogenous glucose in 12-14 month old female *Snell* dwarf mice after glucose injection. Control and *Snell* dwarf mice were injected with glucose (0.8 mg per g body weight) mixed with trace amounts of [^{3}H]glucose (3 x 10^5 cpm per mg glucose). Compared to control mice, plasma glucose was lower in *Snell* dwarf mice at 2.5 5, 10, 15, 20 and 30 minutes after injection, indicating elevated glucose tolerance. Endogenous glucose in plasma was also reduced in *Snell* dwarf mice at 2.5, 5, 10, 15, 20 and 30 minutes after injection, indicating greater inhibition of endogenous glucose production. Results are shown as mean ± SEM. *, ** Significantly different at indicated time-points by nonparametric Student t-test between *Snell* dwarf and control mice where $p<0.05$ and N=6 mice per group.

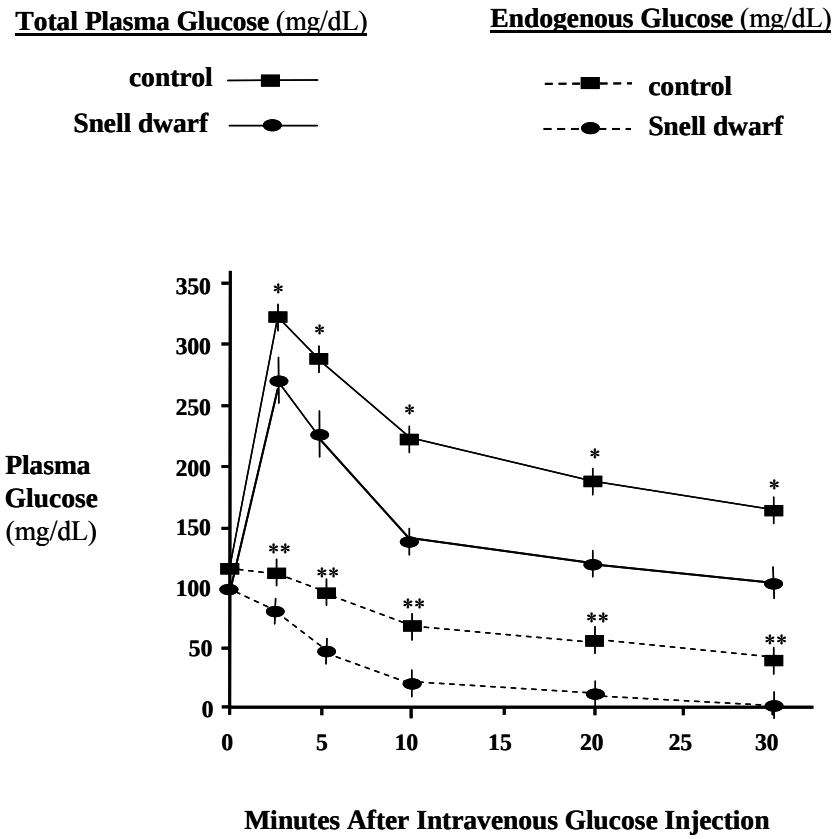


FIGURE 2. Percentage of old female *Snell* dwarf mice with neoplastic lesions at time of death. The percentage of mice with one or more neoplastic lesion at time of death was lower for *Snell* dwarf than control mice. The median age at time of death was 26 months for control and 33 months for *Snell* dwarf mice. Neoplastic lesions identified by histology included histiocytic sarcoma, lymphoma, mammary adenocarcinoma, fibrosarcoma, hepatocellular carcinoma, leiomyosarcoma, myelogenous leukemia and hemangiosarcoma. Bar graphs show results as mean ± SEM. * Significantly different by Chi-square analysis where $p<0.05$ and N=33 control mice and N=11 *Snell* dwarf mice.

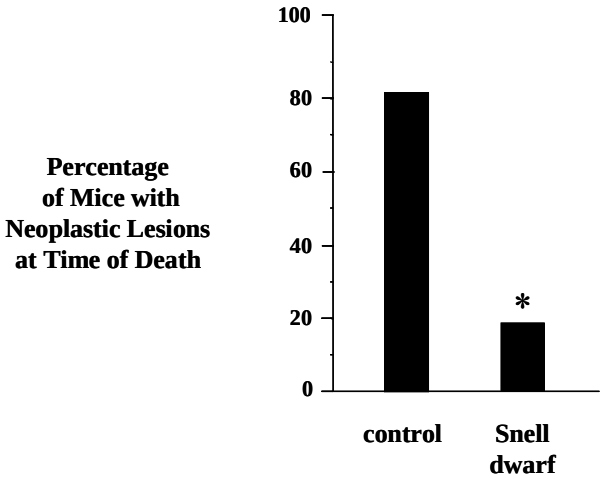


FIGURE 3. Circulating adiponectin in 12-14 month old female *Snell* dwarf mice. Plasma adiponectin was elevated in *Snell* dwarf mice by Western blot analysis. Bar graphs show results as mean ± SEM. * Significantly different by nonparametric Student t-test where $p<0.05$ and N=6 mice per group.

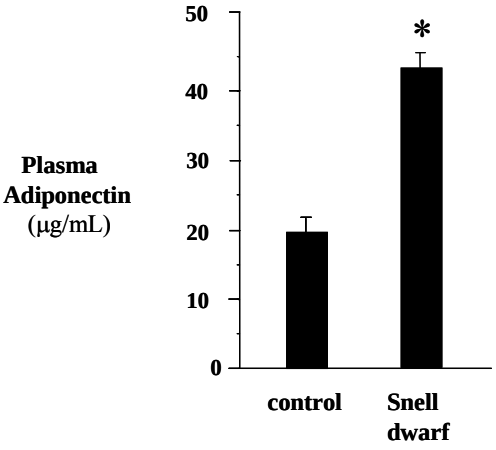
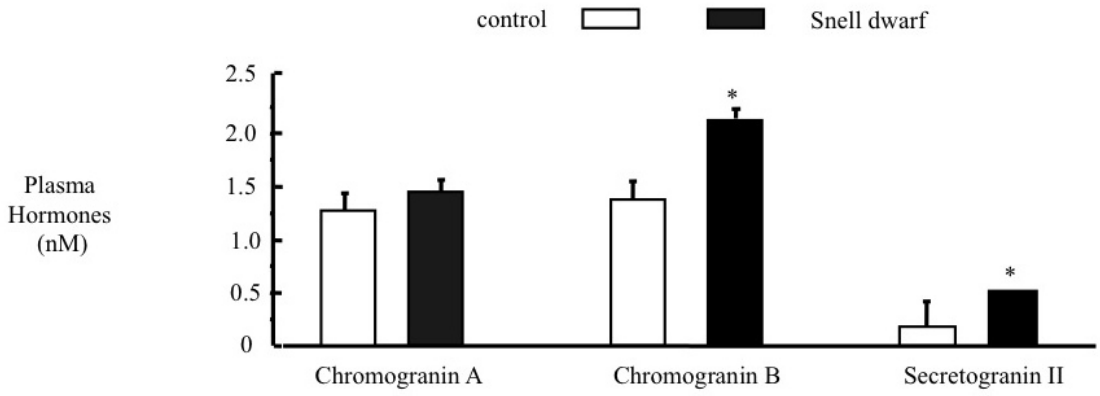


FIGURE 4. (A) Circulating Chromogranin A, Chromogranin B and Secretogranin II in 12-14 month old female *Snell* dwarf mice. Plasma Chromogranin B and Secretogranin II were elevated in *Snell* dwarf mice by radioimmune assay. Bar graphs show results as mean ± SEM. * Significantly different by nonparametric Student t-test where $p<0.05$ and N=15 mice per group for Chromogranin A and N=5 mice per group for Chromogranin B and Secretogranin II. **(B) Tissue Chromogranin B and Secretogranin II mRNA expression by Northern blot analysis in wild-type mice.** Chromogranin B mRNA expression was detected in the pituitary and the adrenal glands while Secretogranin II mRNA expression was detected ubiquitously.

(A)



(B)

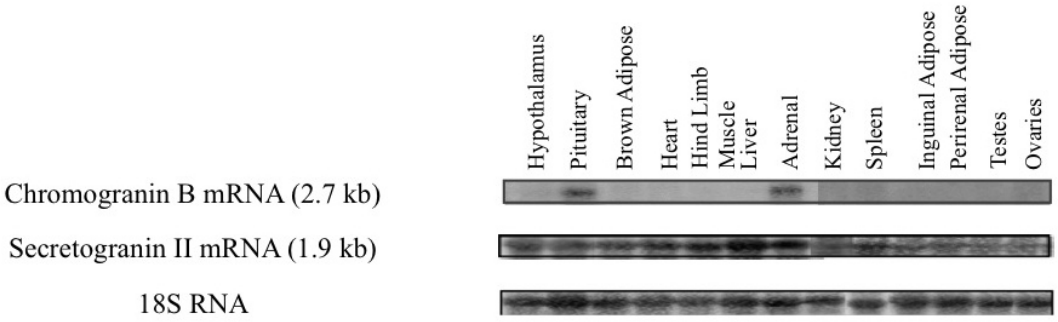


FIGURE 5. Neuroendocrine inhibition of endogenous glucose production and resistance to cancer. *Snell* dwarf mice show elevated adiponectin, Chromogranin B and Secretogranin II and low endogenous glucose production. Furthermore, *Snell* dwarf mice are highly resistant to cancer. Adiponectin, Chromogranin B and Secretogranin II are shown as part of a complex neuroendocrine axis that can ultimately provide resistance to cancer.

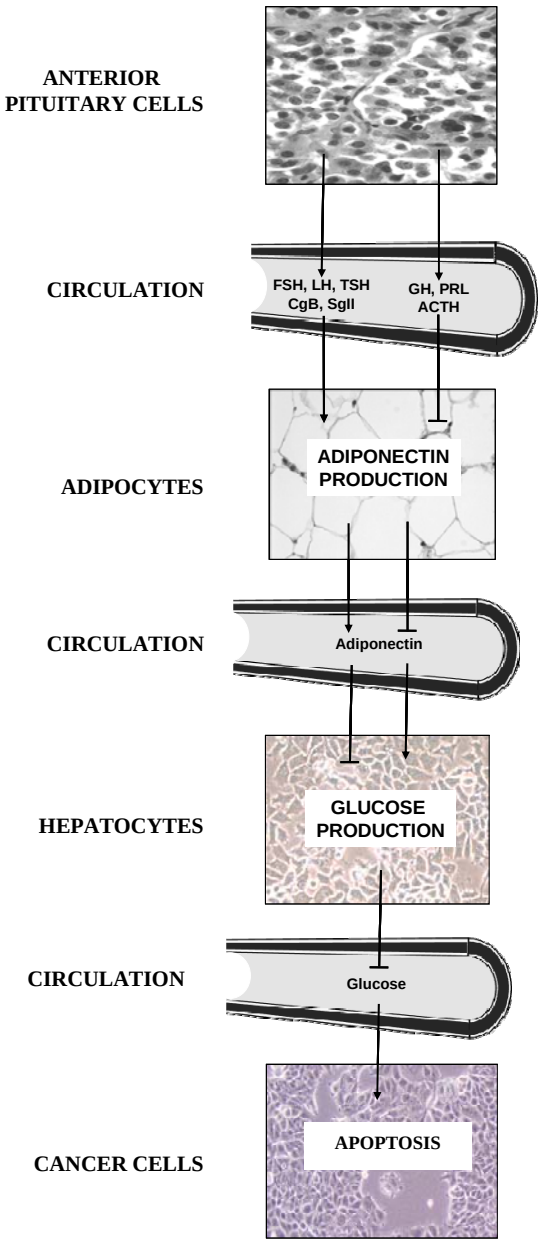


FIGURE 5

DISCUSSION

Our main finding was that inhibition of endogenous glucose production was enhanced after injection of glucose in 12-15 month old female *Snell* dwarf mice in comparison to age-matched controls. Inhibition of endogenous glucose production by injection of glucose simulates the fed state. These results complement previous data that endogenous glucose production is suppressed in *Snell* dwarf mice during fasting (Brooks et al., 2007c).

Snell dwarf and control mice received the same dose of glucose per unit body weight. Although previous studies indicated that *Ames* dwarf mice consume similar amounts of food per unit body weight as control mice, *Ames* dwarf mice show striking differences in glucose metabolism compared to control mice (Argentino et al., 2005; Borg et al., 1995; Dominici et al., 2003; Hauck et al., 2001; Mattison et al., 2000).

Thus, conversion of carbohydrates to fatty acids and the burning of fats may increase longevity compared to immediately burning carbohydrates directly for ATP. Elevated fat utilization for energy, *fat burning*, may be associated with greater fitness than burning of glucose. Low glucose utilization may delay aging by reducing oxidative stress (Brooks et al., 2007c; Nishikawa et al., 2000). Furthermore, the reduction of endogenous glucose production could restrict glucose utilization by cancer cells and inhibit the development of malignant lesions that appear with high frequency in old mice. Diabetes is often associated with elevated glucose production (Basu et al., 2005; Natali and Ferrannini, 2006; Radziuk and Pye, 2002; Stefan et al., 2003; Wajngot et al., 2001) and in a recent clinical study, not a single diabetic colorectal cancer patient showed a complete pathologic response to chemotherapy (Caudle et al., 2008).

Significantly fewer *Snell* dwarf mice had neoplastic lesions at necropsy. Our study showed that only 18% of the *Snell* dwarfs had at least one malignant lesion compared to 82% of the control mice. The low incidence of cancer in *Snell* dwarf mice is in contrast to the high tumor incidence in old *Ames* dwarf mice at time of death (Ikeno et al., 2003). Necropsy revealed that 72% of *Ames* dwarfs showed at least one fatal neoplastic lesion at the time of death compared to 85% in controls (Ikeno et al., 2003).

The mechanisms that provide *Snell* dwarf mice resistance to cancer late in life are obviously mediated by congenital loss of function of the Pit1 gene also called the growth hormone factor-1 gene (GHF1). Pituitary tumors show greater expression of Pit1 than healthy pituitary glands (Asa et al., 1993; Delhase et al., 1993; Sanno et al., 1996). The role of Pit1 as a tumor suppressor may be limited to in somatotrophs (Canibano et al., 2007). Pit1 is elevated in human breast carcinoma compared to normal breast tissue. Pit1 overexpression in MCF7 cells (human breast adenocarcinoma) stimulates GH production, cell growth and cell proliferation (Gil-Puig et al., 2005). Pit1 antisense oligonucleotides reduce GH mRNA expression and [^{3}H]thymidine incorporation in pituitary somatotrophs and lactotrophs suggesting that Pit1 may stimulate DNA replication and cell proliferation (Castrillo et al., 1991). The link between Prop1, the gene mutated in *Ames* dwarf, and cancer is less clear with one study reporting reduced Prop1 mRNA

expression in corticotroph tumors and several studies showing no significant difference and compared with other pituitary tumors.

Catecholamines, glucagon and glucocorticoids are hormones that stimulate endogenous glucose production. Adiponectin, a hormone produced by adipose tissue, inhibits endogenous glucose production (Berg et al., 2001). By inhibiting glucose production, adiponectin may lower whole-body glucose utilization. The elevation of adiponectin may provide resistance to cancer by lowering endogenous glucose production (Fig. 5). The elevation of circulating adiponectin in *Snell* dwarf mice is an endocrine signal that suppresses endogenous glucose production and elevates fatty acid oxidation (Brooks et al., 2007c). This switch in substrate availability may impair cancer cell defense from the immune system and apoptosis.

Glucose utilization may also be reduced in *Snell* dwarf mice by other mechanisms besides the inhibition of endogenous glucose production. *Snell* dwarf mice show elevated insulin sensitivity as indicated by circulating glucose and insulin levels, glucose tolerance tests and insulin tolerance tests (Brooks et al., 2007c; Combs et al., 2003; Mirand and Osborn, 1952; Mirand and Osborn, 1953). The elevation of insulin sensitivity in *Snell* dwarf mice and other long-lived mouse models can lower glucose utilization. Low levels of insulin could lower glucose utilization by reducing GLUT4 mediated insulin transport (Rossetti et al., 1997). Low insulin in *Snell* dwarfs can decrease insulin-mediated utilization of glucose by insulin responsive tissues, mainly muscle and adipose. (DeFronzo et al., 1981; Rossetti et al., 1997) Whether elevated insulin sensitivity in *Snell* dwarf mice is responsible for greater inhibition of glucose production is currently unknown. IGF-I and thyroid hormone deficiency in *Snell* dwarfs also lower tissue demand for glucose (Itoh et al., 2001; Rossetti et al., 1991).

As a first step towards determining the basis for the difference in the incidence of cancer between *Snell* dwarf and *Ames* dwarf mice, we tested whether there are any other pituitary hormone differences in the *Snell* dwarf mice besides a lack of GH, PRL and TSH. Proteomics analysis revealed that pituitary secretion of Chromogranin A, Chromogranin B and Secretogranin II were detected abundantly in *Snell* dwarf mice. Widely differing effects and targets for intact and alternatively processed derivatives of Chromogranin A, Chromogranin B and Secretogranin II have been reported (Helle, 2004). Chromogranin A and Chromogranin B were previously linked to low insulin production which may lower whole-body glucose utilization by reducing GLUT4 mediated glucose transport (Karlsson et al., 2000; Rossetti et al., 1997; Schmid et al., 2007). GLUT4 colocalizes with Secretogranin II in large dense core vesicles (Hudson et al., 1993)**.** The effects of Chromogranin A, Chromogranin B and Secretogranin II on endogenous glucose production are currently unknown. Figure 5 illustrates how the anterior pituitary gland could inhibit endogenous glucose production by regulating circulating adiponectin levels. Endogenous glucose production by the liver is also inhibited directly by the hypothalamus through the hepatic branch of the vagus nerve (Pocai et al., 2005).

The defense mechanisms that suppress the naturally occurring tumors in mice deteriorate during aging. Future studies will investigate the link between glucose metabolism and cancer at an old age. The low incidence of cancer in old *Snell* dwarf mice should be investigated further considering the mechanism may be related to neuroendocrine factors besides than GH and IGF-I. Furthermore, future studies should define the neuroendocrine mechanism(s) that control endogenous glucose production and determine whether the impairment of those mechanisms can provide resistance to the naturally occurring cancers in mice.

CHAPTER II

REVIEW: ANIMAL MODELS FOR GERONTOLOGICAL RESEARCH-
DWARF MICE VS. CALORIC RESTRICTION

ABSTRACT

Gerontological research indicates that the aging process is similar in humans and laboratory rats and mice. What aging process is delayed by calorie restriction (CR) and mutations that produce long-lived dwarf mice? From 1935 until 1996, CR was an exclusive model for increasing lifespan in lab rodents (McCay et al., 1989). In 1996, a mutation producing the *Ames* dwarf mouse was reported to increase lifespan. Additional single-gene mutations causing dwarfism or reducing body weight have been reported to increase longevity in mice since 1996 (Brown-Borg et al., 1996). Long-lived mouse models show elevated insulin sensitivity. Elevated insulin sensitivity reduces oxidative stress, a potential cause of aging. Our data, in the long-lived *Snell* dwarf mouse, suggests that increased adipose tissue production of adiponectin elevates insulin sensitivity, decreases oxidative damage and lowers the incidence of cancer. The elevation of liver insulin sensitivity by adiponectin, can lower oxidative damage and raise cancer resistance by (a) reducing endogenous glucose production and (b) increasing fatty acid oxidation. Future studies on the aging process will focus on the regulation of nutrient metabolism.

CALORIE RESTRICTION

Centenarians escape many ailments associated with aging, such as cancer, stroke, Alzheimer's and heart disease (Perls, 1995). Gerontological research seeks to explain how some people can live over 100 years. The 2-3 year lifespan of the laboratory mouse provides a relatively rapid glimpse of the aging process. Numerous studies show that calorie restriction (CR) causes life extension in laboratory mice and rats (Masoro, 2005; Weindruch, 1996). CR also delays the aging process in humans. The population of Okinawa, which consumes 20% fewer calories than other Japanese populations, has 3.5 times more centenarians and shows a lower incidence of stroke, heart disease and cancer. (Kagawa, 1978) In humans, body weight, fat mass, body temperature, thyroid hormone and oxidative DNA damage decrease after 6 months of 25% CR (Heilbronn, 2006). The reduction of oxidative damage supports that CR retards the aging process by reducing the accumulation of oxidative damage (Beckman and BN, 1998). Regardless of the strong link between CR and longevity, it is cautiously advised, as CR does not increase viability under every type of environmental stress or in every mouse strain (Forster et al., 2003; Gardner, 2005; Keenan et al., 1998).

DWARFISM

Long-lived mutant dwarf mice such as the *Ames* (Proph-$1^{-/-}$), *Snell* (Pit-$1^{-/-}$), and *little* (GhrhR$^{-/-}$) have reduced growth hormone (GH) and insulin-like growth factor (IGF-1) (Brown-Borg et al., 1996; Coschigano et al., 2000; Flurkey, 2001; Flurkey et al., 2002; Kurosu et al., 2005; Migliaccio et al., 1999; Miskin and Masos, 1997). Human Laron syndrome, which is caused by a non-functional GH receptor, analogous to the *little* dwarf mouse, also exhibits reduced GH and IGF-1. However, the human Laron syndrome does not increase lifespan (Laron, 2008). Humans with mutations in the *Prop-1* gene are of shorter stature, like the *Ames* dwarf mouse; however, the small number of human subjects with *Prop-1* mutations did not show a difference in lifespan (Krzisnik, 1999). The contradiction between species could be attributable to the specific mutation, which may not necessarily affect the gene in a similar manner. It should be noted that decreased height has been associated with decreased chronic disease and increased lifespan in population studies (Samaras et al., 2003).

CANCER

The incidence of cancer and death from cancer increase with age because the aging process impairs cellular defense and repair mechanisms (Holliday, 2004). Rous demonstrated over a century ago that underfed or calorie restricted mice that lost weight showed a decrease in the proliferative activity of transplanted tumors. This is based on a delay in the ability of the host to vascularize and form connective tissue necessary for tumor growth (Rous, 1914). CR also reduces the appearance of spontaneous tumors in

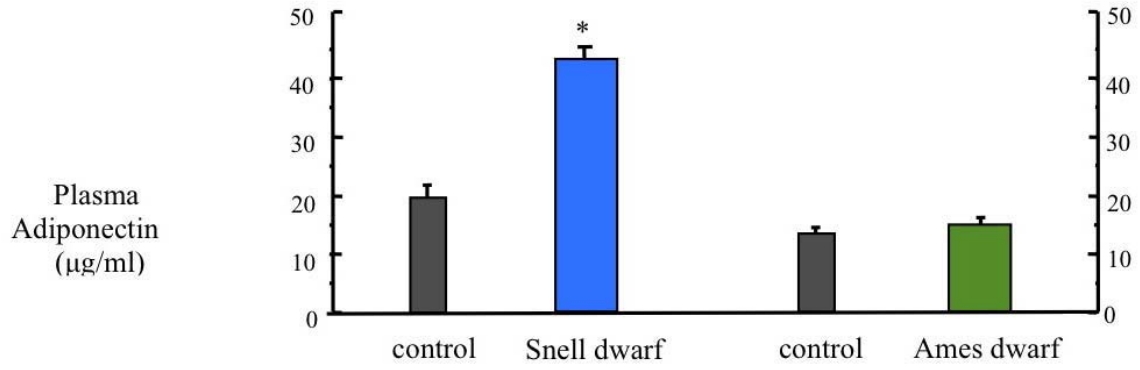
mice. Weindruch et al showed that CR inhibited the growth of spontaneous lymphomas in mice (Weindruch, 1982). Cancer also occurs less frequently in humans on CR (Grifantini, 2008; J*Ames* et al., 1998). The IGF signaling pathway, which is suppressed by CR, promotes tumor growth (D'Costa et al., 1993).

Decreased endogenous glucose production and glucose utilization in *Snell* dwarf mice may account for their resistance to cancer considering that cancer cells depend on glucose for growth (Alderman et al., 2009). Hyperglycemia, an indication of impaired glucose metabolism, is associated with increased risk of cancer in humans (Stattin, 2007). Therefore, age-related changes in nutrient metabolism may be linked to the development of cancer. Akt, an oncogene that promotes cell survival and transformation, stimulates glucose consumption in cancer cells (Elstrom et al., 2004a). In the absence of glucose, these cells are more susceptible to death. Similarly, c-Myc, another oncogene, activates a glucose-dependent pathway and without glucose, c-Myc transformed cells undergo apoptotic cell death (Shim, 1998; Shim et al., 1997). Consistent with increased glycolysis in neoplastic cells, inhibition of certain glycolytic enzymes decreases glucose uptake and reduces tumor growth. Clem et al, demonstrated that inhibition of PFK3B, one of four enzymes that leads to the production of fructose-2, 6-bisphosphate and indirectly activates PFK, reduces tumor growth in mice (Clem et al., 2008). An *ad-lib* low-carbohydrate diet also retarded transplanted colon tumor growth in obese mice (Wheatley et al., 2008).

The absence of GH and low IGF signaling together may contribute to cancer resistance in *Ames* and *Snell* dwarf mice. However, *Ames* dwarf mice show a delay in the appearance of cancer while *Snell* dwarf mice are highly resistant to spontaneous cancer with old age (Alderman et al., 2009). The loss of cancer resistance in old *Ames* dwarf mice may be based on *Ames*/*Snell* differences in (a) background strain or (b) Prop1/Pit1 function. *Snell* dwarf mice exhibit elevated adiponectin levels at middle age, which can reduce endogenous glucose production. This may be due to variances in adiponectin levels. Plasma adiponectin is elevated in young and middle age *Snell* dwarf mice (Alderman et al., 2009; Combs et al., 2003). Plasma adiponectin levels are elevated in young *Ames* dwarf mice; however, the difference disappears by middle age (Figure 6). Life extension by CR in *Ames* dwarf mice may be mediated by the elevation of adiponectin (Bartke et al., 2001).

In humans, type II diabetes is associated with elevated plasma glucose and insulin as well as an increased risk of certain types of cancer. In a recent prospective study, abnormal glucose metabolism, indicated by hyperglycemia, was associated with significantly increased risk for overall cancer in women and pancreatic cancer, malignant melanoma and urinary tract cancers in men (Stattin, 2007). This finding further associates glucose metabolism with the development of cancer, but does not necessarily imply that glucose restriction will help to reduce the risk of cancer in humans.

Figure 6. Circulating adiponectin is similar in middle-aged wild type and *Ames* dwarf mice. (n=5; p>) Young *Ames* dwarf mice have higher plasma adiponectin. The equalization of adiponectin may explain the difference in late life cancer seen in *Ames* dwarf mice (previously unpublished data). Plasma adiponectin was measured by Western blot analysis as previously described and is compared to middle-aged *Snell* and wild type control mice. (Alderman et al., 2009).



OBESITY

Obesity reduces the human lifespan (Flegal et al., 2005). Recent estimates suggest that obesity can decrease life expectancy by as much as seven years (Olshansky et al., 2005; Peeters et al., 2003). Obesity is also associated with increased risk insulin resistance, diabetes mellitus, hypertension, dyslipidemia, coronary heart disease, and certain cancers (Pi-Sunyer, 1993). It is therefore possible that obesity accelerates the human aging process.

Adipose tissue produces substances that regulate nutrient metabolism, energy expenditure, insulin sensitivity, appetite, inflammation, and immunity (Shoelson et al., 2007). The reduction of adiposity has been shown to elicit various beneficial physiological changes. A reduction in adiposity increases insulin sensitivity, decreases hepatic glucose production, serum cholesterol, and blood pressure as well as normalizing plasma adipokine levels, including decreasing plasma C-reactive protein and leptin while increasing adiponectin (Valsamakis, 2004). The reduction of adiposity by CR suggests that the expansion of adipose tissue accelerates the aging process (Bartke et al., 2001; Harrison et al., 1984).

PHYSICAL ACTIVITY

In studies comparing voluntarily-running rats and sedentary rats on CR to keep their body weights the same as those of the runners, CR sedentary controls were long-lived despite having a higher body fat content than runners (Holloszy et al., 1985). Similarly, long-lived male *Snell* dwarf mice become obese and exhibit high plasma levels of leptin late in life (Flurkey, 2001). Despite the late-life obesity and increase in plasma leptin, the *Snell* dwarf is longer lived than control mice. In short, reduced adiposity does not seem to be the primary mechanism involved in the anti-aging effects of CR.

Physical activity is an important modulator of human gene expression influencing lifespan (Booth et al., 2002). The idea that physical activity is critical for human health is consistent with epidemiologic data and is a focus of many public health interventions (Hu et al., 2004). Furthermore, health benefits are said to increase in proportion with increased duration of exercise. While these "health benefits" may include factors such as increased insulin sensitivity, improved cardiovascular health, and weight management, the impact on lifespan is unclear. Data on the effect of physical activity on human longevity is lacking; it cannot be conclusively stated whether exercise confers any benefit on human lifespan.

The hypothesis that linear increases in metabolic rate (and therefore ROS production) result in decreased lifespan suggests that exercise (and the corresponding increase in metabolic rate) would have the same effect. One animal study has shown that voluntary exercise results in increased lifespan while another has shown that voluntary exercise improves survival rates but does not extend lifespan. (Goodrick, 1980; Holloszy et al., 1985). In a study examining the effect of voluntary exercise on longevity, rats engaging in voluntary exercise lived slightly longer than sedentary free-fed and sedentary pair-fed controls, but significantly less long than food restricted paired-weight controls (Holloszy et al., 1985). One study found that, while exercising rats did not live longer than sedentary controls, other markers of aging, such as recent memory retention, were improved with exercising (Samorajski et al., 1985). These results suggest that exercise may have some protective effect that counteracts the increased energy and oxygen utilization (and corresponding oxidative damage) that come with increased physical activity.

While a direct correlation between exercise and length of natural lifespan cannot be concluded, exercise may be an important mediator of the aging process. Exercise improves health outcomes throughout life, enhancing insulin sensitivity and cardiovascular health and improving weight management. Exercise may increase longevity indirectly through weight maintenance.

INSULIN SENSITIVITY

CR may extend lifespan due to increased insulin sensitivity (Al-Regaiey et al., 2007; Holzenberger et al., 2003). It is hypothesized that the CR mice exhibit a redistribution in metabolism, likely away from insulin-dependent signaling, which decreases glucose utilization. However, FIRKO mice, which

lack the insulin receptor in adipose tissue, show a decrease in adiposity and an 18% increase in lifespan (Bluher et al., 2003). The long-lived Klotho mutant, which experiences increased IGF and insulin resistance, is a contradiction to other dwarf mice characteristics (Kurosu et al., 2005). Future studies will need to determine to what degree and in what tissues insulin-like growth factor signaling reductions should take place to delay the aging process.

In humans, obesity is frequently associated with insulin resistance and abnormal glucose homeostasis. Elevated adiponectin, increased peripheral insulin sensitivity and decreased circulating insulin, biomarkers of increased longevity, are evident in centenarians. (Atzmon et al., 2008; Paolisso et al., 2001) The cluster of physiologic changes associated with the metabolic syndrome increases the risk of cardiovascular disease. In adipose tissue, decreased production of the hormone adiponectin can cause obesity, insulin resistance, hyperglycemia and dyslipidemia (Brooks et al., 2007b). Individuals with the lowest plasma adiponectin in a population have the smallest and most dense LDL particles putting them at higher risk of heart disease (Hulthe et al., 2003).

NUTRIENT METABOLISM

CR can exert an anti-aging effect in rodents even when it is implemented at an advanced age (Masoro, 1990a; Masoro, 1990b). CR in humans offers similar positive benefits such as decreased BMI, increased insulin sensitivity, and all factors that decrease risk of diabetes and other health complications (Redman and Ravussin, 2009). *Snell* dwarf mice share multiple characteristics with CR mice: a smaller body size, lower body weight, and extended lifespan (Alderman et al., 2009). These similarities extend to carbohydrate metabolism and food intake. CR mice consume less food than AL mice because less is available to them; *Snell* and *Ames* dwarf mice also consume less food because they require less energy to fuel their small body and slower metabolic activities. Dwarf mice on average exhibit lower plasma glucose and high insulin sensitivity with reduced IGF-1 and TSH. *Snell* dwarf mice and CR mice both show less incidence of cancer than ad lib wild type mice; specifically *Snell* dwarf mice produce less ROS, perhaps decreasing any mtDNA damage (Alderman et al., 2009; Brooks et al., 2007a; Flurkey, 2001). It would seem that the most positive effect on longevity regardless of species would be that of a model with a sparing system.

OXIDATIVE STRESS

Oxidative stress and damage caused by free radicals to proteins, lipids, and DNA, particularly mitochondrial DNA (mtDNA), increases with age in both humans and rodents. Oxidative damage to DNA, lipids, and proteins was measured in muscle biopsy samples from humans 25 to 93 years of age by quantifying the amount of 8-hydroxy-2-deoxyguanosine (8-oxodG), malondialdehyde (MDA), and protein

carbonyl groups as indicators of oxidative damage to DNA, lipid peroxidation, and protein carbonylation, respectively (Mecocci et al., 1993). The results indicate that oxidative damage to DNA, lipids, and proteins in muscle increases with age and suggest that oxidative damage may contribute to aging in human muscle. Oxidative damage was also observed in nDNA and mtDNA in brain tissue from humans 42 to 97 years of age by quantifying the amount of 8-oxodG (Medocci, 1999). The results indicate that oxidative damage increased significantly in both nDNA and mtDNA with age; however the rate of oxidative damage accumulation was much greater for mtDNA. The results from the abovementioned studies suggest that oxidative damage may contribute to aging in humans. Healthy human centenarians with a mean age of 101 years exhibited high levels of plasma vitamin A and vitamin E in comparison to elderly humans 60-99 years of age and young adults less than 60 years of age (Mecocci et al., 2000). This suggests that increased longevity is associated with higher levels of compounds with antioxidant properties such as vitamins A and E which limit oxidative damage.

Lipid, protein, and DNA oxidation was measured in liver tissue of young mice of 5 months and old mice of 18 to 24 months (Colantoni et al., 2001). Old mice showed significantly higher levels of oxidative damage in every measure. The amount of oxidative DNA damage in kidney tissue was found to be 150% higher in old mice, compared to young mice (Fraga et al., 1990). In one study, old rats had approximately 2 million DNA oxidative lesions per cell, compared to only 1 million in young rats (*Ames* et al., 1993). Mice overexpressing the human enzyme catalase in mitochondria displayed increased median and maximum lifespan, as well as, decreased oxidative damage as measured by 8-oxodG, decreased numbers of reactive oxygen species, decreased mtDNA deletions, and reductions in age-related pathologies (Schriner, 2005). These results suggest that neutralizing free radicals could increase longevity by delaying oxidative damage associated aging (Sohal, 1996).

CR decreases the rate of oxidant production, which then results in a reduction in the levels of damaged proteins, lipids, and DNA from age-associated oxidation. Long-term calorie restricted rats displayed significantly less hydrogen peroxide production in liver mitochondria, as well as significantly lower levels of mtDNA damaged by oxidation, compared to rats fed ad libitum. 8-oxodG in old rats age was suppressed by long-term caloric restriction (Lopez-Torres and al., 2002). These results suggest calorie restriction decreases the rate of mitochondrial ROS production and the amount free radical damage to mtDNA in rats.

Dwarf mice display significantly lower levels of oxidative stress than their respective wild type controls. Sanz et.al reported that 8-oxodG in mtDNA from brain tissue was 32% lower in male and 36% lower in female *Ames* dwarf mice; 8-oxodG in mtDNA from heart tissue was 30% lower in male *Ames* dwarf mice, while there were not differences among females (Sanz et al., 2006). The results suggest that,

although sex differences exist, the decreased oxidation in mtDNA in *Ames* dwarf mice may play a role in their increased longevity.

Snell dwarf mice display a significantly different response to oxidative stress than their wild type counterparts. When 3-nitropropionic acid, an inhibitor of succinate dehydrogenase, was used to induce oxidative stress via the production of ROS, *Snell* dwarf mice had less activation of the MEK–ERK kinase cascade, suggesting that this altered tolerance to oxidative stress may play a role in the increased longevity (Madsen et al., 2004). It has also been shown that *Snell* dwarf mice display low glucose utilization and high fatty acid utilization, which is correlated with a low level of oxygen radical damage (Brooks et al., 2007c).

The relationship between free radical damage, oxidative stress, mtDNA damage and longevity is as of yet still unknown. The Free Radical/Oxidative Stress Theory of Aging states: levels of oxidative damage will increase with age; manipulations that increase lifespan will also reduce the levels of oxidative damage; and decreasing levels of oxidative stress will increase lifespan (Bokov et al., 2004). This theory is exemplified in the abovementioned study with glucose metabolism in *Snell* dwarf mice, suggesting that the aerobic metabolism of glucose may lead to more oxygen radical production and more oxidative damage. The idea is based on the altered ratio of NADH to $FADH_2$, which is higher from glucose oxidation compared to amounts of NADH:$FADH_2$ produced from the oxidation of fatty acids (Brooks et al., 2007c). The major producer of reactive oxygen species in the electron transport chain is Complex I where NADH oxidation is the first step and through which flux is greater when glucose is oxidized. Although it is tempting to suggest that directly decreasing oxidative stress in humans will increase longevity, the available evidence does not fully support this theory. More research concerning the relationship between oxidative stress and aging needs to be conducted before recommendations can be made for the human population.

SUMMARY

Gerontological research in human and rodent models suggests that CR delays the aging process. The recent discoveries of single-gene mutant long-lived dwarf mice, which phenotypically mimic CR mice, provide an opportunity to investigate common features between models. Cancer resistance is a common feature in CR and long-lived dwarf models. Increased cancer resistance may be based on the elevation of insulin sensitivity. The hormone adiponectin, produced by adipose tissue, may play a major role in the elevation of insulin sensitivity with CR. Elevated adiponectin and increased insulin sensitivity is also a feature in many long-lived dwarf models. Our recent data suggest that the benefits of elevated adiponectin disappear by middle age in female *Ames* dwarf mice. This may contributes to high incidence of cancer in old female *Ames* dwarf mouse.

Oxygen radical production from the mitochondria is related to energy utilization, a potential cause of aging in lab rodents and humans. The increase of energy expenditure though exercise does not accelerate the aging process. Caloric intake per unit body weight is not altered in CR rats or *Ames* dwarf mice. Elevated insulin sensitivity can alter fuel utilization in a way that decreases oxygen radical production. We conclude that the elevation of adiponectin by CR or the absence of GH in dwarf mice is a major contributor to increased insulin sensitivity. Elevated insulin sensitivity reduces endogenous glucose production and increases fatty acid utilization for energy. ATP produced from fatty acids may generate fewer oxygen radicals than glucose on the basis that glucose oxidation proceeds primarily through complex I whereas fatty acid oxidation is more dependent on complex III. Future studies should focus on mechanisms that will alter nutrient metabolism and potentially delay aging in humans.

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