The Effect of Hypothyroidism on Glucose Tolerance in Dogs

By

Natalie Inteeworn

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
In
Biomedical and Veterinary Sciences

David L. Panciera, DVM, MS, DACVIM
W. Edward Monroe, DVM, MS, DACVIM
Korinn E. Saker, MS, DVM, PhD, DACVN

April 17, 2008
Blacksburg, VA

Keywords: Hypothyroidism, Dog, FSIGT and minimal model analysis, insulin sensitivity, glucose tolerance

Copyright Natalie Inteeworn, 2008

The Effect of Hypothyroidism on Glucose Tolerance in Dogs

by

Natalie Inteeworn

Abstract

Background: Canine hypothyroidism is thought to cause abnormalities in glucose homeostasis, but the effect on glucose tolerance and insulin sensitivity has not been determined to date.

Hypothesis/Objectives: The purpose of the study was to investigate whether hypothyroidism has an effect on glucose tolerance and insulin sensitivity in dogs. We hypothesized that hypothyroidism causes insulin resistance.

Animals: Sixteen euthyroid bitches were randomly selected and allocated into two groups. In 8 dogs, hypothyroidism was induced by administration of 1 mCi/kg ^{131}I. Experiments were performed on non-anesthetized, fasted dogs in anestrous approximately 12 months after hypothyroidism was induced.

Methods: The insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT) and minimal model analysis were used to determine basal insulin and glucose concentrations, acute insulin response to glucose (AIR_g), insulin sensitivity (S_I), glucose effectiveness (S_G) and the disposition index (DI).

Results: In the hypothyroid group, basal glucose concentrations were mildly decreased ($P = 0.0079$), whereas basal insulin was increased ($P = 0.019$). Insulin sensitivity was reduced in the hypothyroid group ($P<0.001$), whereas AIR_g was higher ($P=0.01$). Other parameters were not different between groups.

Conclusions/Clinical Importance: Hypothyroidism negatively affects glucose homeostasis by inducing insulin resistance. In hypothyroid dogs, the disposition index (insulin sensitivity x insulin secretion) remained unchanged due to a compensatory increase in insulin secretion, thereby maintaining glucose tolerance. In cases with impaired insulin secretion, such as canine diabetes mellitus, concurrent hypothyroidism can have important clinical implications in the successful management of the disease.

Acknowledgements

The author wishes to recognize the Virginia Veterinary Medical Association Veterinary Memorial Fund for providing funding for this project, and Dr. W. Edward Monroe and Dr. David Panciera for their work in acquiring the grant.

The author would like to recognize Stephanie Milburn, Barbara Kafka, Ginnie Dreier, Delbert Jones and Dana Calicott for their excellent technical support, as well as Dr. Stephen Werre for statistical analysis.

The author wishes to thank all the members of the graduate committee for their continued assistance throughout this project, and for their support of the author's professional career.

Table of Contents

Abstract .... *ii*

Acknowledgements .... *iv*

Table of Contents .... *v*

Introduction *1*

Chapter I: Literature review *2*

Hypothyroidism 2

Hypothyroidism in dogs 2
Physiology of glucose homeostasis 4
General overview 4
Insulin 5
Glucose transport across cell membranes and insulin signaling 8
Counterregulatory hormones to insulin 12
Growth hormone and Insulin-like growth factors 13
Insulin sensitivity 15
Non-insulin-mediated glucose uptake, glucose effectiveness 15
Glucose tolerance 16
Pathophysiology of insulin resistance and impaired glucose disposal 17

Effect of hypothyroidism on glucose homeostasis 23

Effect in people and in rodent models 23
Effect in dogs 27
Importance 29

Models of estimating insulin sensitivity and glucose effectiveness 30

Chapter II: The effect of hypothyroidism on insulin sensitivity and glucose tolerance *47*

Introduction 47

Materials and Methods 48

Animals 48
Insulin-modified frequently sampled intravenous glucose tolerance test 49
Minimal model analysis 50
Analysis of glucose and insulin 51
Statistical analysis 51

Results 52

Assay Validation 52
Insulin-modified FSIGT and minimal model analysis 53

Discussion **54**

Footnotes *61*

Conclusion *61*

Bibliography *63*

Appendix I: Additional tests *80*

Material and Methods **80**

Growth hormone stimulation and suppression tests 80
Analysis of Growth hormone and IGF-I 81
Urine cortisol and catecholamine concentrations 81
DEXA Scans 82
Statistical analysis 82

Results **83**

List of Figures and Tables *84*

Figures **84**

Figure 1: Average insulin concentrations (+/ SD) during FSIGT in 8 hypothyroid and 7 control dogs 84
Figure 2: Average glucose concentrations (+/- SD) during FSIGT of 8 hypothyroid and 7 euthyroid dogs 85

Tables **86**

Table 1: Correlation coefficients, 95% confidence intervals and P-values for each pair of variables analyzed as described material and methods. Data divided by groups. 86
Table 2: Mean (MN) and standard deviation (SD) per group of additional tests described in Appendix I. 91
Table 3: Data from all analyses as described in material and methods (Chapter II and Appendix I), divided by animal and group. 92
Table 4: Growth hormone (GH) concentrations (in ng/ml) of control and hypothyroid dogs during stimulation and suppression tests as well as peak and nadir GH concentrations, differences (absolute and percent) of peak and nadir to average (AVE) basal GH concentrations, and area under the curve (AUC). 94

Introduction

Naturally occurring hypothyroidism is a common endocrine disorder of the adult dog with reported prevalences of 0.2% and 0.64% (Milne and Hayes 1981; Panciera 1994 a). A variety of clinical, biochemical and hematological abnormalities have been identified, but are common with many diseases and are very unspecific in nature. Besides commonly recognized clinical abnormalities such as alopecia, pyoderma, weight gain and lethargy, hypothyroidism has also been linked to various other diseases and abnormalities. These include peripheral and central nervous system dysfunction, myopathy, cardiovascular abnormalities and abnormal reproductive function (Jaggy et al. 1994; Panciera 1994 a; Panciera 1994 b; Panciera et al. 2007). Hypothyroidism has also been implicated to cause insulin resistance, and diabetes mellitus was the most common concomitant disorder in one retrospective study (Dixon et al. 1999).

Glucose homeostasis is a tightly regulated system maintaining serum glucose concentrations in a narrow range. Insulin is the most important hormone responsible for glucose disposal. It promotes glucose uptake in insulin-sensitive tissues, causes inhibition of hepatic glycogenolysis and gluconeogenesis, inhibits lipolysis of adipose tissue and promotes triglyceride synthesis. Glucagon, cortisol, growth hormone (GH) and catecholamines are considered hormones that counterbalance insulin actions and increase blood glucose levels under fasting conditions. Growth hormone exerts much of its effects through insulin–like growth factors, including insulin-like growth factor I (IGF-1), which plays a role in glucose homeostasis.

Insulin resistance is a result of impaired insulin action on insulin sensitive sites, mainly muscle, fat and hepatic tissue, which increases the risk of glucose intolerance. In humans,

the pathogenesis is usually multifactorial, but genetic and acquired factors play important roles. In conjunction with declining β-cell function, it may lead to overt diabetes mellitus. Many diseases in dogs are reported to cause insulin resistance (Peterson 1995). Based on a very limited number of clinical, retrospective reports (Ford et al. 1993; Dixon et al. 1999), experimental studies (Renauld et al. 1973) and personal clinical observation, there is evidence that hypothyroidism leads to insulin resistance. In humans, hypothyroidism has been implicated to alter insulin and glucose metabolism and cause impaired glucose tolerance and insulin resistance (Clausen et al. 1986; Pedersen et al. 1988; Rochon et al. 2003).

Therefore, the aim of the present study was to evaluate the effect of hypothyroidism on glucose tolerance and insulin sensitivity. In addition, concentrations of insulin-antagonizing hormones and IGF-I concentrations were determined and potential associations with parameters of glucose disposal assessed. Lastly, dual energy x-ray absorptiometry (DEXA) scans were performed to evaluate differences in body composition between groups with special emphasis on percent body fat and distribution.

Chapter I: Literature review

Hypothyroidism

Hypothyroidism in dogs

Hypothyroidism is one of the most common endocrine disorders in the dog. Naturally occurring hypothyroidism is an adult-onset, acquired disease usually affecting middle aged to older dogs. In the majority of cases, it is a primary disorder of the thyroid gland

(primary hypothyroidism), but can, less commonly, stem from a failure of the pituitary gland to produce thyroid-stimulating hormone (TSH) (secondary hypothyroidism). Congenital, iatrogenic and nutritional hypothyroidism and hypothyroidism secondary to neoplastic destruction of the thyroid gland are rare but recognized presentations. Histologically, primary hypothyroidism is characterized by an irreversible destruction of the thyroid follicles, which function as a production and storage site of thyroxine (T4) and triiodothyronine (T3). The two most common causes for destruction and failure of the thyroid gland to function normally are lymphocytic infiltration leading to autoimmune or lymphocytic thyroiditis and idiopathic follicular atrophy, in which case thyroid parenchyma has been replaced by fibrous or adipose tissue. An association between the two with follicular atrophy being the end stage of lymphocytic thyroiditis has been discussed, but not clearly proven to date.

Breed predilection, heritability and sex predisposition for the female gender are reported, and neutering was found to be an additional risk factor (Milne and Hayes 1981; Panciera 1994 a). Clinically, affected dogs show relatively unspecific signs such as lethargy, weight gain, exercise intolerance, weakness, bradycardia and dermatological abnormalities. such as seborrhea, alopecia and pyoderma most commonly seen amongst others (Panciera 1994 a; Dixon et al. 1999). Neurological abnormalities reported to be associated with hypothyroidism include localized neuropathies such as vestibular and facial nerve paralysis as well as generalized neuropathies (Jaggy et al. 1994) and central vestibular disease (Higgins et al. 2006). Changes in myocardial function and electrical activity are common, but usually subtle and of no clinical significance. One study evaluating echocardiographic and electrocardiographic parameters in hypothyroid dogs

frequently identified mild left ventricular function impairment, bradycardia, weak pulses and low voltage electrocardiogram complexes, whereas arrhythmias such as first degree atrioventricular block were seen less frequently (Panciera 1994 b). Reports related to reproductive dysfunction due to hypothyroidism have been controversial. A recent report found no change in fertility, although prolonged parturition and reduced pup survival was observed in experimentally induced hypothyroid mixed breed bitches (Panciera et al. 2007). Ocular abnormalities such as corneal lipidosis and chronic uveitis are thought to be rare and induced by hyperlipidemia secondary to hypothyroidism (Crispin and Barnett 1978; Kern and Riis 1980). Biochemical and hematological abnormalities can include hypercholesterolemia, hypertryglyceridemia, hyponatremia, decreased concentrations of inorganic phosphate, elevated creatine kinase, alkaline phosphatase, aspartate aminotransferase and γ-glutmayltransferase activities and a mild nonregenerative anemia (Milne and Hayes 1981; Panciera 1994 a; Dixon et al. 1999).

Physiology of glucose homeostasis

General overview

The major function of carbohydrates is to serve as a energy source and function as precursors of essential intermediates for use in synthetic processes. Amongst those, the monosaccharide glucose plays an important and central role in energy metabolism and homeostasis in mammalian cells. Certain cell types require a constant supply of this principal nutrient, thereby being dependent on glucose as their primary energy source than others. In the dog as well as other species, erythrocytes and neurons in the brain are examples of cells that preferentially use glucose as their energy substrate (Kaneko et al.

1997). Severe hypoglycemia can have devastating consequences, with seizures, loss of consciousness and even death being the most severe. Conversely, persistent hyperglycemia is known as diabetes mellitus and represents a common disease in both humans and companion animals. Life-threatening complications can arise and develop if the hyperglycemic state is left untreated. In addition, both hyperglycemia and hypoglycemia in the critically ill have been associated with adverse outcomes (Bruchim et al. 2006; Nasraway 2006; Krinsley and Grover 2007; Torre et al. 2007). Therefore, blood glucose levels need to be regulated within a relatively narrow physiological range. A complicated interplay between several hormones and organ systems exists to accomplish this goal, and the contribution of each hormone will be discussed.

Insulin

Insulin is produced by the β-cells of the pancreatic islets of Langerhans. These cells synthesize preproinsulin on the rough endoplasmic reticulum, where it is subsequently cleaved to proinsulin. Proinsulin is a single-chain looped polypeptide composed of 78 amino acid residues in the dog, which corresponds to a molecular weight of about 9,000 Dalton (Da). The structure contains disulfide linkages to bond certain amino acids together. After synthesis, the prohormone is transported and stored in the secretory granules of the Golgi apparatus, where proteolytic enzymes cleave a central connecting polypeptide or C-peptide from the chain. The two remaining chains, now called A and B chain are still linked by disulfide bonds, and the whole monomeric molecule represents the final form of the hormone insulin. The A chain contains 21 and the B chain 30 amino acid residues with a molecular weight of about 6000 Da. Four molecules of insulin monomers normally form a tetramer, which is the active form. Amino acid sequences

differ slightly amongst species, but other than having an effect on the immunological behavior, it does not affect biological activity. Plasma half-life of insulin is about 5-10 minutes and degradation sites are the liver and, to some extent the kidneys and muscles (Kaneko et al. 1997). The primary stimulus of insulin release and further production is glucose, whose blood levels typically increase after food intake and subsequent absorption from the gastrointestinal tract. There are many other stimuli that either directly increase insulin secretion or potentiate the glucose stimulus for insulin secretion. These include certain gastrointestinal hormones (gastrin, secretin, cholecystokinin, gastric inhibitory peptide), amino acids, especially arginine and lysine, β-adrenergic stimulation and other seemingly counterregulatory hormones to insulin (see below), such as glucagon, growth hormone, cortisol and, to a lesser extent, progesterone and estrogen.
In addition, certain drugs such as sulfonylureas (e.g. glipizide, glyburide, tolbutamide) and isoproterenol are known to stimulate insulin release as well. Inhibitory signals are hypoglycemia, fasting, somatostatins, alpha-adrenergic stimulation and leptin, an adipocytokine from peripheral fat tissue (Guyton and Hall 2000).

Glucose typically triggers a multiphasic insulin secretory response. The first peak is very quick, reflecting the release of a readily mobilizable pool of insulin, whereas the following peaks (usually at least one or two more) correspond to the release of granules from a reserve pool (Bratanova-Tochkova et al. 2002).

In general, insulin can be viewed as a hormone that is associated with energy abundance, helping to utilize and store energy in different parts of the body. These effects can be subdivided into three categories, namely the effects on carbohydrate, fat and protein metabolism. After a carbohydrate-containing meal, blood glucose levels increase and

insulin is secreted. It facilitates glucose uptake into muscle cells, which are relatively impermeable to glucose under resting conditions and promotes preferential use of glucose as an energy source over fatty acids. Moreover, insulin causes excess glucose to be converted into glycogen, a storage form of glucose, while enzyme activities for gluconeogenesis are inhibited. The liver is the primary storage organ for glycogen, followed by muscle tissue. Insulin promotes uptake of glucose into the liver cell by increasing enzyme activities of glucokinase and glycogen synthase, thereby increasing the rate of phosphorylation of glucose and subsequent glycogen synthesis. At the same time, insulin inhibits phosphorylase, an enzyme whose primary function is the breakdown of glycogen into glucose. Once glycogen concentration reaches about 5 to 6 %, the liver converts excess glucose into fatty acids and then into triglycerides, which are then transported to adipose tissue and deposited as fat. All these processes ensure that excess glucose is stored until it is needed during fasting, when blood glucose levels decrease again. The effect of insulin on carbohydrate metabolism in other cells depends on the tissue of origin. Most of the remaining cells in the body will increase the uptake and utilization of glucose in response to insulin, similar to muscle and liver cells. Some cells, on the other hand, take up glucose constitutively and are therefore insulin-independent. Examples are brain cells, erythrocytes and kidney cells. This depends largely on the tissue distribution of different glucose transporters, which will be discussed later.

The effect of insulin on fat metabolism is very important and complex as well. The overall effects are fat synthesis and storage, as well as inhibition of lipolysis. As previously mentioned, insulin promotes glucose uptake by liver cells and conversion into fatty acids and triglycerides. Insulin also facilitates glucose uptake into adipose tissue

cells, where most of the absorbed glucose is converted into α-glycerol phosphate, the substrate for the glycerol moiety of the triglyceride molecule. Insulin promotes storage of fat by inhibiting the action of hormone-sensitive lipase, an enzyme that enhances hydrolysis of already stored triglycerides.

Lastly, insulin also affects protein metabolism. Excess glucose after a meal is stored as glycogen and fat, but also used for protein synthesis and storage. Insulin promotes transport of specific amino acids into cells where they are used for translation of mRNA into protein precursors. Additionally, insulin will increase transcription of selected DNA sequences into mRNA, increasing the availability of certain mRNA molecules to be translated.

All the above-mentioned processes are basically reversed when insulin is not present, corresponding to the fasting condition. Previously stored nutrients are once again available as an energy substrate. Muscle cells will first deplete glycogen stores and then use fatty acids as a substrate. Simultaneously, gluconeogenesis and glycogenolyis ensure glucose supply from the liver. Loss of inhibition of hormone-sensitive lipase in adipose tissue causes release of fatty acids and glycerol into the circulation. Amino acids are also released into the circulation from protein catabolism, ultimately resulting in increased muscle wasting. Hormones involved in these metabolic processes will be discussed later.

Glucose transport across cell membranes and insulin signaling

While glucose disposal is mostly insulin-dependent in the absorptive state, it is insulin-independent under fasting conditions or the postabsorptive state. During fasting, hepatic glucose output is the major source of glucose, which is primarily used by brain cells. As already mentioned, there are tissues such as neurons and erythrocytes that are more

reliant on glucose as their major energy source than others, and it is those cells that constitutively absorb glucose, independent of the presence or absence of insulin. The dependency on insulin for glucose uptake is determined by a tissue-specific distribution of different types of glucose transporters.

Cell membranes, with their lipid bilayer, are not permeable to simple glucose diffusion, therefore cells need specific glucose-transporting proteins. In recent decades, two distinct molecular families of glucose transporters have been identified and cloned. One is the sodium-linked glucose cotransporter (SGLT) that actively transports glucose against a concentration gradient. Such transporters are highly restricted to the brush border membrane of intestinal cells and proximal tubular cells of the kidney (Bell et al. 1990). The other family of glucose transporters is referred to as the facilitative type of glucose transporters (GLUT). They are not coupled to any energy-requiring steps and allow bi-directional movement of glucose across the cell membrane along a chemical gradient (facilitative diffusion). Glucose transporters are present in all mammalian cells and are specific to the D-isoform of the glucose molecule. Many isoforms of the facilitative glucose transporters have been identified to date, some of which have well defined functions, whereas the role of others is not yet as clear. There are five well-described, major homologous transmembrane transporter proteins, namely GLUT 1 - 5. While sharing sequence homologies, the transporters differ in their substrate specificities, transport kinetics and tissue distribution, which ultimately dictate their functional role (Gould and Holman 1993; Olson and Pessin 1996; Shepherd and Kahn 1999). GLUT 1 is ubiquitously expressed on cells with particularly high levels in fetal tissues, fibroblasts,

erythrocytes and endothelial cells of the brain. GLUT 2 protein is a low affinity glucose transporter found on the basolateral surfaces of liver cells, pancreatic β-cells, small intestine and kidney. In the pancreas, this is the glucose transporter that senses increased blood glucose levels after a meal, which subsequently stimulates insulin release. GLUT 3 is expressed primarily in neuronal tissue such as brain and is considered the major glucose transporter responsible for glucose transport into the brain and peripheral nerves. Together, GLUT 1 and 3 allow glucose to cross the blood-brain barrier and enter neurons. GLUT 5 is expressed in the apical brush border of intestinal cells and has been shown to have a high affinity for another hexose sugar, fructose. Two other genes (GLUT 6 and 7) have been identified as well, but one is a pseudogene-like sequence and is not expressed as a protein (GLUT 6) and the function of GLUT 7 is not as clear as previously thought (Burchell 1998). New facilitative glucose transporter proteins have been identified in recent years (GLUT 8-13) (Rogers et al. 2002; Manolescu et al. 2007), but none have been functionally characterized to the level of detail obtained with GLUT 1-5.

Finally, the GLUT 4 is unique from all the other glucose transporters in that it is the main insulin-responsive glucose transporter protein. It is expressed in skeletal and cardiac muscle as well as white and brown adipose tissue. The mechanism by which insulin increases glucose transport kinetics is unique as well. While other GLUT proteins are permanently located in the cell membrane, about 90% of GLUT 4 is sequestered intracellularly in distinct vesicles. In response to insulin, but also other stimuli such as exercise or muscle contraction, vesicles translocate to the plasma membrane and to the transverse tubules in muscle tissue, where they fuse with those membranes, increasing the number of available GLUT 4 proteins. While the Michaelis-Menten constant k_m does not

change by this process, the maximal velocity (V_{max}) of glucose transport into the cell is significantly increased. Studies in rat adipose cells have shown that insulin causes a 20-30-fold increase in glucose transport, whereas it is only 2-4-fold in human adipose cells. In muscle cells of rats and humans, transport is increased 7- and 2-fold, respectively. Even though adipose tissue has insulin-responsive glucose transporters, the main site for more than 80% of glucose disposal is muscle tissue. It is therefore considered to be most important in terms of impaired glucose uptake in insulin resistance. Once insulin concentrations decline, GLUT 4 transporters are internalized by endocytosis making them available for later use. This recycling provides a rapid and efficient system to insulin and, indirectly, glucose fluctuations in the blood (reviewed in (Gould and Holman 1993; Shepherd and Kahn 1999; Watson and Pessin 2001)).

Insulin-mediated GLUT 4 translocation occurs through activation of multiple complex signaling pathways. Amongst those, the phosphoinositide-3-kinase (PI 3-kinase) pathway is the best-studied and most important. Binding of insulin to the extracellular portion of a transmembrane insulin receptor protein activates tyrosine kinase phosphorylation at the intracellular portion of the receptor. This subsequently activates insulin-receptor-substrate molecules (IRS) and other substrates, which then activate PI 3-kinase. Formation of 3' phosphoinositide activates further downstream protein kinases such as phosphinositide-dependent protein kinase (PDK) and protein kinase B (PKB or Akt) and protein kinase C (PKC) isoforms, which eventually mediate GLUT 4 translocation. The complete signaling cascade and the individual importance of several already identified protein kinases has not been fully elucidated. There is at least one additional insulin receptor signaling pathway independent of the PI 3-kinase pathways, such as exercise-

induced activation of GLUT 4 translocation that may involve 5'-AMP-activated kinase (Watson and Pessin 2001).

Counterregulatory hormones to insulin

Several counterregulatory hormones to insulin increase glucose concentration when it falls below normal plasma concentration. Glucagon, a polypeptide released from α-cells of the pancreatic islets is thought to be the main counterregulatory hormone. Its metabolic effects are opposed to those of insulin. Glucagon promotes hepatic glucose output by increasing glycogenolysis and gluconeogenesis, activates adipose cell lipase to provide fatty acids for energy, and inhibits triglyceride storage in the liver. Blood glucose concentration represents the major regulator of insulin and glucagon secretion. A decrease of blood glucose concentration below the normal range increases plasma glucagon secretion several-fold. High concentrations of specific amino acids (alanine and arginine) and exercise also stimulate secretion of glucagon.

Growth hormone from the anterior pituitary gland, cortisol from the adrenal cortex and epinephrine from the adrenal medulla also play important roles in glucose homeostasis. Growth hormone and cortisol are released in response to hypoglycemia, although their release is not as acute as insulin and glucagon secretion. They both cause inhibition of cellular utilization of glucose and promote fat utilization. Epinephrine release as a result of sympathetic nervous system activation, increases plasma glucose concentration by enhancing glycogenolysis. At the same time, plasma fatty acids are increased due to a direct lipolytic effect by activating hormone sensitive lipase (Guyton and Hall 2000).

Growth hormone and Insulin-like growth factors

Growth hormone (GH) is a polypeptide hormone that is secreted primarily from the anterior pituitary gland. Other tissues that secrete GH in humans are mammary gland, brain, lymphocytes, pineal gland and placenta. In addition, secretion from mammary tissue in response to progestins has been documented in dogs (Selman et al. 1994). In the pituitary, secretion is primarily controlled by the stimulatory growth hormone releasing hormone (GHRH) and the inhibitory hormone somatostatin, but many other factors have been identified to affect GH secretion and action (Butler and Roith 2001). Secretion is pulsatile and shows sexual dimorphism in humans and rodents. Male rats have been shown to have high amplitude episodic secretory bursts, while female rats show a more continuous secretion with lower peaks and higher baseline values (Eden 1979; Jansson et al. 1985). The largest and most consistent surge in humans occurs at the onset of sleep. One study in healthy male dogs identified GH secretory bursts rhythmically every 4.5 hours (French et al. 1987), while another study found 1 to 3 peaks every 6 hours (Cowan et al. 1984). Yet another study could only identify seasonal but no circadian or ultradian fluctuation in healthy female and male dogs (Gobello et al. 2002).

Growth hormone is an anabolic hormone that enhances amino acid uptake and protein synthesis in skeletal muscle. It impairs glucose uptake in tissues such as skeletal muscle and fat and increases glucose production by the liver. These effects are associated with insulin resistance. One study in which GH was administered to healthy male adult humans associated pronounced insulin resistance with hyperinsulinemia and a postreceptor defect in insulin signaling (Rosenfeld et al. 1982). Furthemore, GH increases fat mobilization and utilization for energy, thereby causing an increase in fatty acid and

glycerol levels in the circulation. The increased plasma fatty acid concentrations may cause impaired insulin action on tissue glucose utilization, an effect that is also known as the Randle cycle (Randle et al. 1963; Butler and Roith 2001). The other extreme, GH deficiency, has also been associated with insulin resistance (Johansson et al. 1995; Hew et al. 1996; Carroll et al. 1998), which may be related to an increase in central adiposity and decreased peripheral glucose utilization due to an inhibition of the glycogen synthase pathway in skeletal muscle (Hew et al. 1996; Carroll et al. 1998).

Growth hormone is thought to mediate most of its effects on metabolism and growth through other growth factors, namely insulin-like growth factors. Amongst those, insulin-like growth factor-I (IGF-I or somatomedin-C) is thought to be the most important one and is mostly produced by the liver. The IGF family includes three ligands (IGF-I, IGF-2 and insulin), six binding proteins (IGFBP-1 through 6) and cell surface receptors (IGF-I receptor, insulin receptor and IGF-II mannose-6-phophate receptor). In circulation, insulin-like growth factors are bound to IGF-binding proteins (mostly IGFBP-3), which act as carrier proteins prolonging the half-life of IGF molecules. Plasma concentrations are therefore stable throughout the day, in contrast to GH levels. Growth hormone stimulates not only synthesis of IGF, but also of IGFBPs. The GH/IGF system is characterized by a classical negative feedback loop, with high IGF-I levels suppressing further GH secretion (Hartman et al. 1993). While the original somatomedin hypothesis in the 1950s suggested that GH acts primarily on the liver where it stimulates IGF-I synthesis and release, it was later discovered that IGFs are expressed in almost all tissues, therefore having autocrine and paracrine effects and that local IGF production was mediated through GH as well. (Le Roith et al. 2001).

Insulin-like growth factor-I has growth-promoting effects during both embryonic and postnatal growth and levels correlate with body size in dogs and humans (Gourmelen et al. 1984; Rijnberk et al. 2003). As the name implies, it has similar actions as insulin by increasing protein metabolism and enhancing glucose uptake into peripheral tissues. In this regard, IGF-I and GH have opposing effects, suggesting that certain effects of GH occur independently of IGF-I.

Insulin sensitivity

Insulin sensitivity is defined as the ability of a given amount of insulin to exert its physiological actions. The higher the sensitivity, the less insulin is needed to normalize glucose concentrations. This glucose-lowering effect is mediated through glucose uptake and suppression of hepatic glucose production. In this context, impaired insulin action is also referred to as decreased insulin sensitivity or insulin resistance, terms that are used interchangeably (Matthaei et al. 2000).

Non-insulin-mediated glucose uptake, glucose effectiveness

There are three insulin-independent components of glucose uptake. Glucose uptake by neurons is not only independent of insulin, but independent of glucose concentrations as well. Other cells of the body, such as hepatocytes, red blood cells and renal medullary cells increase glucose utilization with increasing glucose concentrations. Likewise, certain tissues such as skeletal muscle are able to increase glucose uptake in an insulin-dependent and insulin-independent fashion. The ability of glucose, per se, to enhance its own disappearance independent of a dynamic insulin response is called glucose effectiveness. The role of glucose effectiveness has received less attention than insulin

sensitivity and secretion, but its importance is being increasingly recognized. An early study utilizing a dog model concluded that by suppressing a dynamic insulin response with somatostatin the effect of glucose per se is at least as important as the dynamic insulin response to the normalization of glucose (Ader et al. 1985). This effect was confirmed with different models and under dynamic and steady state conditions (Ader et al. 1997). Importantly, glucose effectiveness involves not only increased utilization of glucose by mass action, but also suppression of endogenous production. Taken together, insulin action and glucose effectiveness both contribute to glucose tolerance and can be considered to act synergistically on glucose utilization, with insulin accelerating glucose disappearance.

Glucose tolerance

Glucose tolerance is the ability of different tissues to dispose of glucose and to maintain normal blood glucose values. It is determined by a complex interaction of numerous factors, including insulin sensitivity, β-cell function (i.e. the degree of insulin secretion), glucose effectiveness, insulin clearance and insulin-antagonizing hormones. Any alteration of this interaction can progress to subclinical, impaired glucose tolerance (IGT) or to overt glucose intolerance and type 2 diabetes mellitus. Together with impaired fasting glucose (IFG), impaired glucose tolerance is one of two criteria used in human medicine to describe an intermediate metabolic state between normal and diabetic glucose homeostasis, also known as the prediabetic state. A general consensus exists that impaired glucose tolerance and decreased β-cell function have to co-exist in order to cause overt diabetes (Bergman et al. 2002). A reciprocal relationship between insulin secretion and insulin action has been described in a hyperbolic function (Bergman et al.

1981). As long as a decline in insulin sensitivity or action can be compensated for by a reciprocal and adequate increase in insulin secretion, glucose tolerance is maintained. The product of insulin sensitivity and secretion has been named the disposition index, which represents another measure of glucose tolerance; as long as it can be held constant, glucose tolerance is maintained.

Pathophysiology of insulin resistance and impaired glucose disposal

Insulin resistance can be viewed as a state of impaired or subnormal insulin action on its target sites. Most of what is known about the pathophysiology of insulin resistance is derived from human medicine, especially in relation to type 2 diabetes mellitus. The clinical spectrum of insulin resistance in humans varies from subjects that have normal glucose tolerance despite insulin resistance to a subgroup with impaired glucose tolerance and finally those with overt type 2 diabetes mellitus. The first two stages are also known as prediabetic states and are accompanied by hyperinsulinemia to compensate for the insulin resistance. In the early stage, this compensatory response is sufficient to maintain glucose tolerance and normal fasting and postprandial glucose levels. With the development of impaired glucose tolerance, hyperglycemia may eventually develop. A concomitant decline in β-cell function, which occurs with the progression through the different stages, is thought to ultimately lead to overt type 2 diabetes. Importantly, even though insulin secretion declines, the resultant insulin deficiency is relative, and basal insulin levels are usually normal or increased in subjects with type 2 diabetes (reviewed in (Matthaei et al. 2000)).

Many factors affect the development of insulin resistance. In humans, it is thought to primarily be an inherited feature with a polygenic inheritance pattern in most cases,

although acquired factors also play an important role in the progression to glucose intolerance. Twin studies, familial clustering of type 2 diabetes, and the high prevalence of this disease in some ethnic groups have shown substantial evidence that type 2 diabetes and insulin resistance are inherited (Matthaei et al. 2000). For example, the extremely high prevalence of type 2 diabetes in Pima Indians in Arizona is worth mentioning, because it strikingly exemplifies the strong genetic association. About 35% of the population are affected by the disease with a 3.9 times higher risk when both parents are diabetic (Knowler et al. 1981). This represents the highest incidence of type 2 diabetes in the world. Other conditions leading to insulin resistance in humans are pregnancy, glucocorticoid use, or endocrinopathies in which counterregulatory hormones to insulin are elevated. Examples are Cushing's syndrome, acromegaly, glucagonoma, and pheochromocytoma, but overt diabetes only develops in a subset of these patients. Certain other diseases in humans are known to be associated with insulin resistance, such as the metabolic syndrome and polycystic ovary syndrome. Importantly, chronic hyperglycemia itself is known to lead to impaired insulin secretion and sensitivity and is also referred to as glucose toxicity (Rossetti et al. 1990). Finally, in rare cases, severe insulin resistance can be caused by autoimmune disorders producing autoantibodies to the insulin receptor, or by specific genetic defects in the insulin-signaling cascade, for example insulin receptor mutations (Matthaei et al. 2000; DeGroot et al. 2001).

Insulin resistance in type 2 diabetes mellitus occurs at various sites, but muscle tissue, liver, adipose tissue and, to a lesser degree, kidneys are believed to play important roles. In muscle and to some lesser degree adipose tissue, insulin resistance is caused by an impaired insulin-stimulated glucose uptake, while decreased ability of insulin to suppress

hepatic (and renal) endogenous glucose production accounts for hepatic insulin resistance. Adipose tissue plays a minor role in glucose uptake compared to muscle tissue. It contributes to insulin resistance mainly from an impaired inhibition of lipolysis with subsequent increase of plasma free fatty acids (FFA) and glycerol. This in turn directly affects both muscle and liver metabolism, as the increased availability and utilization of FFA's inhibits glucose uptake and oxidation and stimulates endogenous glucose production. In addition, glycerol serves as a gluconeogenic substrate to the liver (reviewed in (Matthaei et al. 2000)).

Obesity and physical inactivity are major contributory factors to insulin resistance. Weight loss and exercise clearly improve insulin sensitivity. Six weeks of exercise training in normal and insulin resistant offspring of type 2 diabetic patients significantly increased glucose phosphorylation and glycogen synthesis in muscle tissues, thereby improving insulin sensitivity in both groups (Perseghin et al. 1996). Studies in the insulin resistant, obese Zucker rat revealed contraction-induced stimulation of GLUT 4 glucose transporter translocation and increased glucose transport activity, suggesting improved glucose uptake into muscle cells (Henriksen 2002). Another study in isolated perfused rat hindlimb muscle showed that glucose transport was increased secondary to contractions. This subsequently increased blood flow and improved availability of insulin in target tissues (Hespel et al. 1995). Increased body fat and other lipid abnormalities contribute to the development of insulin resistance and type 2 diabetes (Ferrannini et al. 1997; Holland et al. 2007). The role of free fatty acids was already discussed earlier. Overactivity of the sympathetic nervous system in obese human subjects and type 2 diabetic patients has been implicated to be one mechanism leading to elevated FFA concentrations (Hilsted et

al. 1987; Scherrer et al. 1994). Special attention is being paid to body fat distribution, as abdominal fat accumulation in particular negatively affects glucose and lipid metabolism (Bergman et al. 2007; Holland et al. 2007). The results of the Insulin Resistance Atherosclerosis Study suggest that abdominal obesity (versus general obesity) predicts future declines in insulin sensitivity in normoglycemic, non-obese individuals, potentially increasing their risk of diabetes (Karter et al. 2005). Gastaldelli et al. recently highlighted the negative impact of abdominal adiposity (visceral and hepatic) on FFA and glucose metabolism, by primarily increasing hepatic insulin resistance (Gastaldelli et al. 2007). A recent review by Bergman et. al supports the portal theory of insulin resistance, in which FFA's from visceral fat enter portal circulation, thereby impairing insulin action (Bergman et al. 2007).

The precise molecular mechanisms of insulin resistance are not known, but abnormalities in the insulin-signaling chain are believed to contribute to the pathogenesis. For example, reduced autoactivation status of the insulin receptor from skeletal muscle and adipocytes has been described. Decreased expression and phosphorylation level of early insulin signaling elements downstream of the insulin receptor (i.e., IRS, PI-3 kinase, PKB) have been demonstrated in insulin target tissues of type 2 diabetic patients, which may ultimately contribute to insulin resistance (reviewed in (Matthaei et al. 2000)). Mutations of one or both insulin receptor alleles can occur in rare cases and cause severe insulin resistance syndromes in people (e.g., leprechaunism, Rabson-Mendenhall syndrome). Several other insulin receptor mutations have been identified in type 2 diabetic people, but those defects were uncommon and have shown to mildly affect signaling function. In

the search for candidate genes for insulin resistance, many other heterozygous mutations of genes that are involved in insulin signaling have been identified, but in most cases, these mutations are not sufficient to cause insulin resistance. However, it is unknown whether a combination of those mutations, perhaps concomitant with other risk factors such as obesity, ultimately leads to insulin resistance and overt type 2 diabetes. This would be in agreement with the postulated polygenic pathogenesis of type 2 diabetes (reviewed in (Matthaei et al. 2000)).

Noteworthy, albeit poorly understood in the regulation of glucose tolerance, glucose effectiveness is thought to potentially play a substantial role for glucose uptake when insulin action and secretion are impaired. One study demonstrated that glucose effectiveness remains normal in the type 2 diabetic state, which could therefore be a primary determinant of total glucose uptake (R_d) in this disease (Alzaid et al. 1994). Another study showed that the relative contribution of non-insulin mediated glucose uptake markedly increases in insulin resistant states such as type 2 diabetes (Baron et al. 1985). This hyperglycemia-mediated increase in R_d is believed to occur by mass action as well as activating key rate-limiting enzymes such as glycogen synthase in the liver (Kruszynska et al. 1986) and even by glucose-mediated translocation of the GLUT 4 transporter into the plasma membrane (Galante et al. 1995).

In dogs, a number of diseases are reported to cause insulin resistance (Peterson 1995), including iatrogenic or naturally occurring hyperadrenocorticism, pancreatitis, ketoacidosis and other concurrent illnesses or infections. These diseases are associated with elevated concentrations of one or more insulin antagonizing hormones, namely

cortisol, glucagon and possibly epinephrine. However, unlike in humans, these conditions alone may not cause overt diabetes, unless pancreatic β-cell function is diminished, for example due to immunological destruction of the β-cells or chronic pancreatitis. Therefore, in order to maintain glucose tolerance, most dogs with normal pancreatic function are able to compensate for the insulin resistance by increasing insulin secretion. In cases where type 1 diabetes is already established, insulin resistance resulting from concurrent diseases will render diabetic control more difficult. Less commonly, growth hormone hypersecretion leads to the clinical picture of acromegaly, which is frequently associated with insulin-resistant diabetes mellitus. In dogs, GH excess usually originates from the mammary gland secondary to overproduction or supplementation with progestagens and is therefore more common in females (selman, mol, 1994).

Although obesity causes hyperinsulinemia and insulin resistance in dogs (Mattheeuws et al. 1984), no published data have demonstrated that obesity is a risk factor for canine diabetes. This may again be related to the fact that dogs are not reported to develop a form of diabetes analogous to type 2 diabetes, such as in humans or cats. On the other hand, the dog has been proven to be a particularly useful model to study the strong association between visceral obesity and insulin resistance in humans (Mittelman et al. 2002; Kim et al. 2003; Bergman et al. 2007).

Underlying molecular mechanisms in association with diseases that lead to insulin resistance in dogs have been extrapolated from human medicine. Generally, a defect in insulin binding to its receptor, decreased numbers of insulin receptors, or impaired postreceptor insulin signaling are suspected. One study in dogs has actually shown that

hyperadrenocorticism causes hyperinsulinemia and decreased erythrocyte insulin receptor binding (Wolfsheimer and Peterson 1991).

Effect of hypothyroidism on glucose homeostasis

Effect in people and in rodent models

Thyroid hormone status affects glucose metabolism in people and animals. Interestingly, both hyperthyroidism and hypothyroidism have been associated with impaired glucose tolerance. In this regard, hyperthyroidism has been associated with insulin resistance as well as decreased insulin-stimulated glucose disposal or increased hepatic glucose production (Bratusch-Marrain et al. 1985; Dimitriadis et al. 1985; Karlander et al. 1989). Other clinical studies documented that there is either no effect on glucose metabolism or insulin sensitivity (McCulloch et al. 1983; Randin et al. 1986) or even increased insulin-stimulated glucose disposal at euglycemia (Muller et al. 1986) or hyperglycemia (Bratusch-Marrain et al. 1984).

Even though less numerous, similar results have been obtained in humans when evaluating the effect of hypothyroidism on glucose metabolism. One recent study, using the euglycemic hyperinsulinemic clamp technique in 6 hypothyroid human patients, before and after hormone replacement therapy, showed that overall glucose uptake was decreased in patients with untreated hypothyroidism. Basal glucose and insulin levels were similar, but despite using the same rate of insulin infusion during the clamp, plasma insulin concentrations were significantly higher in the untreated hypothyroid group compared to after hormone replacement therapy, presumably due to changes in insulin

half-life. These findings not only support other studies in that glucose metabolism in humans is dependent on thyroid status, but also that hypothyroidism decreases insulin action and sensitivity (Rochon et al. 2003). Likewise, an impaired maximal insulin effect on glucose oxidation was documented when insulin action was studied in adipose tissue fragments from 6 hypothyroid patients, and a strong negative correlation between thyroid hormone level and insulin receptor number on fat tissue cells (r = -0.72) was found. The results are consistent with impaired insulin responsiveness and glucose metabolism due to impaired insulin action at the postreceptor level, as insulin binding and numbers were markedly increased in hypothyroid patients (Arner et al. 1984). Similarly, Pedersen and coworkers studied insulin receptor binding, glucose transport and glucose oxidation, using isolated adipocytes that were obtained from 6 untreated hypothyroid, 10 hyperthyroid and 10 healthy, euthyroid patients. For both diseased groups, insulin resistance was documented. In particular, hypothyroidism was associated with reduced insulin receptor binding at low insulin concentrations, impaired insulin sensitivity of glucose transport and decreased maximal insulin responsiveness of glucose transport and lipogenesis rates. These results demonstrated that thyroid hormones affect insulin action at receptor and postreceptor sites (Pedersen et al. 1988). It was concluded that the comparison of the discrepant results regarding insulin receptor binding compared to the study by Arner et al. was not valid, as data was obtained by a different analysis, and experiments conducted at different temperatures and different insulin concentrations in culture media (Pedersen et al. 1988). Another study, using the euglycemic hyperinsulinemic clamp, Stanicka et al. demonstrated significantly lower parameters of insulin sensitivity in 15 thyroidectomized female patients than in the same patients during

hormone-replacement therapy. Moreover, fasting levels of cortisol, glucagon, epinephrine and growth hormone were significantly elevated in the untreated hypothyroid state, although correlations to insulin sensitivity markers did not reach statistical significance (Stanicka et al. 2005). Another clinical study demonstrated that the presence of subclinical hypothyroidism in patients with concurrent rheumatoid arthritis (n = 14) remained an independent predictor of insulin resistance, as defined by higher Homeostasis Model Assessment ($HOMA_{IR}$) and lower Quantitative Insulin Sensitivity Check Index (QUICKI) values compared to euthyroid controls (n = 97) (Dessein et al. 2004). In contrast to these results, when HOMA was used to estimate insulin sensitivity in 22 patients that had undergone total thyroidectomy and radioiodine ablation, hypothyroidism had no impact on insulin sensitivity (Owecki et al. 2006). Similarly, despite the finding of fasting hyperinsulinemia in patients with subclinical hypothyroidism (n = 77), insulin sensitivity based on HOMA-IR was normal and not different from the control group (Tuzcu et al. 2005). The disparity in data for these studies could be related to the fact that, in all three studies, HOMA was an inappropriate test choice to assess insulin sensitivity in regards to study size, as the test is only considered accurate in larger epidemiological or cohort studies (Wallace et al. 2004). Lastly, no difference was found in 10 hypothyroid patients compared to euthyroid controls when evaluating fractional and metabolic clearance of insulin and glucose alone. Insulin administration in this group caused glucose concentrations to stay below baseline throughout the test period, indicating that insulin action was not opposed by the presence of hypothyroidism. Disadvantages of the study were the choice of test and small sample size (Shah et al. 1975). Similar to Shah, a study published by Clausen showed that iv-

administration of insulin in hypothyroid patients causes a more profound hypoglycemic reaction (Clausen et al. 1986), implying that hypothyroid patients are more insulin sensitive. The results obtained with this study design are not well comparable to more accepted models, assessing insulin action and glucose tolerance under more physiological situations, i.e., after glucose stimulation.

The pathophysiological processes involved in the development of insulin resistance are thought to be different for both hyperthyroidism and hypothyroidism and have mostly been evaluated in laboratory animals or *ex vivo* studies. As shown by DNA microarray studies on hepatocytes, many enzymes involved in gluconeogenesis are positively regulated by thyroid hormone (Feng et al. 2000), thereby opposing the action of insulin on hepatic glucose production and leading to hepatic insulin resistance. In addition, hyperthyroidism has been shown to increase insulin clearance in people, which may be a factor leading to a decreased insulin action due to faster disappearance (Dimitriadis et al. 1985; Randin et al. 1986). Conversely, it has been shown that thyroid hormone increases glucose turnover and insulin-mediated glucose disposal in rat skeletal muscle and mouse adipose cells (Weinstein et al. 1991; Romero et al. 2000), which is in line with improved insulin sensitivity at those sites.
In contrast, hypothyroidism-associated insulin resistance may primarily be related to decreased skeletal and adipose tissue sensitivity to insulin, thus resulting in decreased glucose utilization (Cettour-Rose et al. 2005). In rats, a T_3-responsive element has been found in the gene encoding insulin-sensitive glucose transporter (GLUT 4). This in turn leads to increased GLUT 4 expression and a subsequently increased glucose transport in

insulin-sensitive tissues in the presence of hyperthyroidism and, conversely, a decrease in glucose transport in hypothyroidism (Weinstein et al. 1991; Dimitriadis et al. 1997; Torrance et al. 1997). Furthermore, in isolated rat muscle, thyroid hormone deficiency has been associated with decreased rates of glycolysis and lower lactate production, further demonstrating a decrease in insulin-stimulated rates of glucose utilization (Dimitriadis et al. 1989; Dimitriadis et al. 1997). Importantly, as hypothyroidism has been found to cause a decrease in hepatic gluconeogenesis and glycogenolysis (Goldblatt 1936; Okajima and Ui 1979; Comte et al. 1990), an associated decrease in glucose disposal may not be clinically relevant. Results of a more recent study, employing the euyglycemic hyperinsulinemic clamp in hypothyroid rats, suggest that insulin resistance may, at least in part, be related to lowered plasma leptin and potentially increased resistin levels (Cettour-Rose et al. 2005).

Effect in dogs

Few studies evaluating the effect of hypothyroidism on glucose homeostasis in dogs have been performed. Only three publications link hypothyroidism with insulin resistance in canine patients. One clinical report describes three diabetic dogs that were evaluated for suspected insulin resistance. This was defined as difficulty or inability of diabetic control despite receiving a dose of >1.5 U/kg of an intermediate-acting insulin or inability in diabetic control with a previously effective dose (1.3 U/kg). Successful treatment of concurrent hypothyroidism markedly improved glycemic control and allowed a reduction of insulin dosage (Ford et al. 1993), suggesting that this was the underlying cause for insulin resistance. Another study investigated epidemiological, clinical, hematological

and biochemical characteristics of 50 dogs with naturally occurring hypothyroidism and found 10% to have concurrent diabetes mellitus. Immune-mediated destruction of both glands and/or the presence of insulin resistance were suggested to be possible underlying mechanisms (Dixon et al. 1999). Finally, when evaluating concurrent disorders in dogs with diabetes mellitus, hypothyroidism was amongst the most common diseases identified, being present in 4% of the dogs (Hess et al. 2000).

Only a few experimental studies have evaluated the effect of abnormal thyroid function on insulin sensitivity in dogs. All available information in the English literature originated from the same research group, and methodologies were very similar in each of the published studies: Hyper- or hypothyroidism was experimentally induced by either chronic administration of l-thyroxine or by ^{131}I-treatment, respectively. Responses to intravenous or oral glucose administration, similar to a glucose tolerance test, were evaluated by measuring plasma glucose and endogenous insulin concentrations at multiple time points. Hyperthyroidism evoked a significantly lower insulin secretory response, whereas blood glucose concentrations remained significantly higher ranging from 40 minutes to the end of the test at 150 minutes. These results indicated impaired glucose utilization as well as impaired insulin response. But whether impaired insulin secretion or increased insulin degradation may be the cause for the latter finding was not determined (Renauld et al. 1974). Hypothyroidism, on the other hand, consistently resulted in exaggerated insulin responses in response to hyperglycemia (Renauld et al. 1972; Renauld et al. 1973; Renauld et al. 1974), whereas blood glucose profiles differed slightly between studies. When IVGTTs were performed, no change in glucose profiles and Conard’s k constant as a measure of glucose disappearance was observed (Renauld et

al. 1972; Renauld et al. 1973), while OGTTs generally resulted in a definite delay to return to baseline and an exaggerated hyperglycemic response, respectively (Renauld et al. 1973; Renauld et al. 1974). In all three studies, the abnormal insulin responses were normalized by treatment with l-thyroxine. In summary, results of those studies demonstrate impaired insulin responsiveness and insulin resistance in hypothyroid dogs, but importantly, a standardized method to estimate insulin sensitivity had not been used.

Importance

Little is known about the effect of hypothyroidism on insulin sensitivity and other parameters of glucose disposal in dogs. The importance of such knowledge is most obvious when looking at the results published by Ford et al. (Ford et al. 1993), in which 3 diabetic dogs seemed to have been difficult to manage until hypothyroidism was corrected by hormone replacement therapy. In addition, as results of two retrospective studies imply, hypothyroidism is not an uncommon concurrent disorder in dogs diagnosed with diabetes mellitus (Dixon et al. 1999; Hess et al. 2000). Therefore, successful management, i.e. glycemic control, of dogs that are already diabetic may be impeded by the concurrent presence of hypothyroidism that possibly caused insulin resistance. Moreover, hypothyroidism may cause subclinical impaired glucose tolerance, which in conjunction with developing diabetes mellitus could lead to or exacerbate complications that may be associated with the disease, the worst one being diabetic ketoacidosis.

Models of estimating insulin sensitivity and glucose effectiveness

A variety of different models estimate insulin sensitivity and other parameters of glucose disposal. Some are relatively simple to perform and interpret and are therefore more suited for the routine clinical setting, whereas other models are more complex and remain restricted to clinical research. In general, the easier the performance of the test method chosen, the more limitations there are to its accuracy in estimating insulin sensitivity.

Fasting plasma insulin concentration is an easy, inexpensive and practical test to perform, and elevated insulin concentrations are thought to reflect the presence of insulin resistance. This assessment does not take into account that insulin concentration depends on insulin sensitivity as well as insulin secretion, distribution and degradation. While it may be an accurate predictor of insulin sensitivity in normoglycemic individuals, it does not provide accurate estimates in patients with abnormal ß-cell function, as seen with impaired glucose tolerance or diabetes mellitus. Other, quite commonly applied models incorporate fasting plasma glucose levels in a formula for a better estimate of insulin sensitivity. Amongst those, Homeostasis Model Assessment ($HOMA_{IR}$) as well as Quantitative Insulin Sensitivity Check Index (QUICKI) are frequently used, simple and inexpensive models, especially in population-based or epidemiological studies. Low $HOMA_{IR}$ and high QUICKI values reflect high insulin sensitivity. The $HOMA_{IR}$ does not give an accurate estimate of insulin sensitivity in patients with severe hyperglycemia or in subjects with ß-cell dysfunction, and it seems to be most useful for the evaluation of insulin sensitivity in euglycemic patients or those with mild diabetes. Compared to $HOMA_{IR}$, QUICKI uses the reciprocal of the logarithm of both glucose and insulin to

account for a skewed distribution of fasted insulin values. As expected, there is a very good correlation between the $HOMA_{IR}$ and QUICKI, especially when $HOMA_{IR}$ is log-transformed. QUICKI may be applicable over a wider range of insulin sensitivity than $HOMA_{IR}$. Both share the same inaccuracies and weaker correlation to values obtained by the frequently sampled intravenous glucose tolerance test (FSIGT) or the hyperinsulinemic euglycemic clamp (see below) when ß- cell function is diminished and insulin levels are low (summarized in (Monzillo and Hamdy 2003; Trout et al. 2007)). The oral glucose tolerance test (OGTT) is one of the most commonly performed tests to assess carbohydrate metabolism in clinical situations in humans. There are several variations of this test in terms of glucose dose and sampling times. After a standardized oral glucose load, the test assesses plasma glucose disappearance at certain time points. By reaching or exceeding defined glucose concentrations per time point, the patient is considered to be normal, have impaired glucose tolerance or overt diabetes mellitus (DeGroot et al. 2001). By measuring both glucose and insulin concentrations at each time point of the test (usually at 0, 30, 60, 120 minutes), it is also possible to estimate insulin sensitivity. A variety of indices (Monzillo and Hamdy 2003) derived from different formulas with data from the OGTT have been published, and all share good correlation with insulin sensitivity determined by the euglycemic clamp. The OGTT is considered a less costly and labor-intensive procedure compared to both the FSIGT and the euglycemic clamp, and seems practical for epidemiologic studies and for population screening. As with $HOMA_{IR}$ and QUICKI, it provides an inadequate estimate of insulin sensitivity in insulin deficient conditions, such as with type 2 diabetes and in patients with impaired glucose tolerance. Other disadvantages of the OGTT include the lack of

sufficient suppression of hepatic glucose production, the influence of gut hormones and neural stimulation on insulin secretion (in addition to pancreatic ß-cell function), the influence of gastric emptying and splanchnic glucose uptake on plasma glucose concentrations and, finally, the poor reproducibility of the test. More detailed summaries of different models and tests and their correlations to clamp and FSIGT (see below) have been reviewed elsewhere (Monzillo and Hamdy 2003; Trout et al. 2007).

The American Diabetes Association Consensus Conference on insulin resistance has accepted two methods that satisfactorily assess insulin resistance, namely the frequently sampled intravenous glucose tolerance test (FSIGT) with minimal model analysis and the euglycemic hyperinsulinemic clamp (EHC) (American Diabetes Association 1998). In 1966, Andres and coworkers developed the clamp technique to study the effect of hyperglycemia on parameters that are involved in glucose homeostasis, and DeFronzo et.al. subsequently standardized the technique in 1979.

The euglycemic clamp is regarded as the gold standard to quantify insulin sensitivity in vivo. It is widely used in the research setting and is highly reproducible (DeFronzo et al. 1979). During the test, insulin is infused into the circulation at a fixed dosage and constant rate to raise plasma insulin concentration acutely to a new plateau, where it is maintained for 120 minutes. The level that is achieved, approximately 100 μU/ml above basal in people, would cause profound hypoglycemia, but euglycemia is maintained by infusion of variable rates of glucose. Under steady state plasma glucose conditions, the amount of glucose infused must equal the amount of glucose being translocated out of the glucose space, provided that endogenous glucose production is completely suppressed.

The rate of glucose infusion (GINF or INF) that is needed to maintain euglycemia at steady state (i.e., glucose metabolized (M), in [mg x (kg x min)$^{-1}$] is an indicator of insulin sensitivity. The more glucose that is needed to maintain euglycemia, the more insulin sensitive the individual is. Importantly, the model assumes that basal hepatic (i.e., endogenous) glucose production is completely suppressed by the exogenous administration of both glucose and insulin. Using [3-^{3}H] glucose, it as been demonstrated that a 100 μU/ml increment in plasma insulin, as used in the EHC, decreases hepatic glucose production to less than 10-15% of basal levels (DeFronzo et al. 1978). Prager and Olefsky showed that steady state suppression of hepatic glucose output is complete in normal subjects, whereas it was only ~80% suppressed in obese study objects (Prager et al. 1986). Therefore, in cases where hepatic glucose production may be less suppressed, such as in states of insulin deficiency or insulin resistance, the value of M will underestimate the total amount of glucose metabolized. In addition, the value of M is dependent on ambient glucose concentrations, which may make comparison between individuals difficult (Bergman et al. 1989)

In addition to the euglycemic clamp, the so-called hyperglycemic clamp technique is considered the gold standard to assess β-cell function (i.e., endogenous insulin secretion) (Elahi 1996). As with the EHC, the hyperglycemic clamp places the plasma glucose level under the investigator's control, but aims at creating hyperglycemia instead of euglycemia. While the EHC mainly assesses tissue sensitivity to insulin, the hyperglycemic clamp also evaluates ß-cell sensitivity to glucose as well. Hyperglycemia is created (approximately 125 mg/dl above basal) with empirical priming and a

subsequent computed maintenance dose of glucose infusion. Insulin concentrations are concurrently determined during the duration of the test (every 2 minutes for the first 10 minutes, then every 10 minutes for two hours). Plasma insulin response (I) to fixed hyperglycemia is a measure of the ß-cell response to glucose. Endogenous glucose production is assumed to be completely suppressed as well, and the rate of glucose infusion reflects the body's glucose utilization and tissue sensitivity to endogenously secreted insulin. For the hyperglycemic clamp, a small correction for urinary losses of glucose (UC) has to be made to calculate M, and a so called space correction (SC) has to be applied to both the euglycemic and hyperglycemic clamps to account for the fact that plasma glucose concentrations cannot be maintained perfectly:

M = INF– SC – UC (in hyperglycemic clamp only), expressed in [mg/(kg x min]

M in the hyperglycemic clamp studies essentially represents a measure of glucose tolerance, but the so called M/I ratio as a measure of the quantity of glucose metabolized at steady state per unit of endogenously secreted insulin concentration is used as a reasonable index of tissue sensitivity to insulin (i.e., insulin sensitivity).

The parameter for insulin sensitivity derived from the EHC (M) is considered more reliable than the M/I ratio (DeFronzo et al. 1979), as all of the infused insulin is known to be biologically active, whereas a small amount of insulin secreted (and measured) during the hyperglycemic clamp is proinsulin. Nevertheless, there is a statistically significant correlation when M/I is plotted against M from the euglycemic clamp. A more detailed description of the methods and calculations is found in the original publication by DeFronzo et.al. (DeFronzo et al. 1979).

The so-called glucose clearance, also obtained during the EHC, has been advocated by some investigators as an index of insulin sensitivity (Ader and Bergman 1987). It is defined as the ratio of glucose utilization rate (R_d) to the concurrent glucose concentration (G), or as R_d/G. The relationship between R_d and G turned out to be non-proportional (Gottesman et al. 1984), with declining R_d/G ratios as glucose levels increase. Comparison of values can therefore be difficult between individuals with different fasting glucose concentratiosn or if individuals are clamped at different glucose levels.

Due to the pitfalls associated with the use of M during the EHC (especially the dependence on ambient glucose), M/I ratio or glucose clearance, the use of an insulin sensitivity index has been introduced ($S_{IP(clamp)}$), using data obtained by the EHC. $S_{IP(clamp)}$ basically represents the slope of the relationship between glucose clearance (see above) and insulin. The slope is the change in glucose clearance divided by the increment in plasma insulin (Bergman et al. 1989):

$$\text{Slope} = ((R_d/G)_e - (R_d/G)_b)/\ I_e - I_b$$

$(R_d/G)_e$ equals steady-state glucose clearance at hyperinsulinemia (I_e), whereas $(R_d/G)_b$ represents glucose clearance at basal insulin (I_b).

The above equation can be used as the basis for calculation of the insulin sensitivity index, requiring glucose clamps to sequentially increment insulin concentrations to achieve different plasma insulin concentrations, while maintaining the same glucose level

(G). By doing so, glucose uptake (R_d) and insulin concentrations (I) can be measured at each plasma insulin concentration, serving as the basis for calculation of $S_{IP(clamp)}$:

$S_{IP(clamp)} = \Delta R_d/G \times \Delta I$ [dl/(min x m^2) per μU/ml] (Bergman et al. 1987)

This parameter is the steady-state ratio of the increment in glucose uptake (ΔR_d) to the increment in plasma insulin concentration (ΔI), normalized to the ambient plasma glucose concentration (G) at which the clamp is performed. In other words, it represents the *change* in clearance ($\Delta R_d/G$) per unit *change* in insulin and should be, on a theoretical basis, independent of glucose concentration (Ader and Bergman 1987; Bergman et al. 1989).

Even though considered the gold standard, there are some limitations to the euglycemic clamp. The contribution of non-insulin-mediated glucose uptake (NIMGU) on total glucose uptake (M), which was discussed earlier, is relatively small in normal individuals studied at hyperinsulinemia (Bergman et al. 1985), but its relative contribution increases markedly in insulin resistant states such as type 2 diabetic subjects (Baron et al. 1985; Alzaid et al. 1994). Continuing to use M values without consideration of this important confounding factor therefore leads to underestimation of insulin resistance (Bergman et al. 1989). Because of this, Bergman and colleagues suggested using the insulin sensitivity index from clamps, $S_{IP\ (clamp)}$, as it is independent of NIMGU and it has the potential for not changing over the physiological range of plasma insulin and plasma glucose concentrations (Bergman et al. 1989). Second, insulin action under steady-state

conditions of the clamp differ from conditions under which insulin acts normally, basically lacking a dynamic assessment of insulin action. Furthermore, plasma insulin concentrations achieved during the EHC exceed insulin levels in plasma (and therefore in the remote insulin action compartment, where insulin action occurs) achieved under normal feeding conditions. Steady state conditions during the clamp are needed to measure a simple index of insulin action (M), but steady state hyperinsulinemia is not achieved in the remote compartment under normal physiological conditions where insulin action occurs, such as after a meal, or even after oral or intravenous glucose tolerance tests. Insulin action under the steady state of a clamp is therefore not representative of the conditions under which insulin is secreted and it does not detect dynamic defects in insulin action that may contribute to insulin resistance (Prager et al. 1986). Lastly, despite offering exquisite precision for the parameters it measures, clamp studies are labor intensive, technically difficult to perform, and expensive.

In 1979, because of the lack of a test that could assess dynamic insulin action as an important factor in the assessment of insulin sensitivity, Bergman introduced the frequently sampled intravenous glucose tolerance test (FSIGT). It represented a modification of the conventional intravenous glucose tolerance test that was being used at the time (Bergman et al. 1979). In contrast to the oral glucose tolerance test, results of the intravenous glucose tolerance test are not influenced by gastrointestinal factors (e.g. absorption, hormones, motility). Limitations of the data derived from the simple intravenous glucose tolerance test were the lack of enough data-sampling points to enable a meaningful analysis and the inability to separate glucose and insulin kinetics from the

results. The FSIGT is based on what is called the minimal model of glucose and insulin kinetics. It is the simplest mathematical representation that, in contrast to the static euglycemic clamp, allows for a dynamic approach to the assessment of insulin sensitivity, while at the same time including the smallest possible number of physiologically meaningful parameters to still account for the complex insulin and glucose dynamics (Bergman et al. 1979; Toffolo et al. 1980). For the FSIGT, approximately 26 to 30 blood samples are obtained during a 3-hour period, with the majority of samples taken from 20 minutes before to 30 minutes after a bolus of dextrose has been given. During each time point, glucose and insulin concentrations are determined, which will then be used for the minimal model analysis. Modeling yields a measure of insulin sensitivity from the influence of endogenous (or exogenous, see below) insulin on the pattern of recovery of basal glucose after intravenous glucose injection. Model assumptions are that glucose disappearance is directly dependent on insulin concentrations in a compartment remote from plasma (Bergman et al. 1979). Plasma insulin (I(t)) enters the remote compartment, where it acts to inhibit hepatic glucose production and stimulate peripheral glucose disappearance. In addition, glucose also accelerates its own disappearance independent of insulin, known as glucose effectiveness. The model also allows for the inhibitory effect of insulin on hepatic glucose balance (Bergman et al. 1979). Parameters of the model are estimated from the least-squares fitting of the glucose data. The minimal model consists of two equations that account for the relationship between the time course of plasma insulin and the resulting pattern of glucose in plasma. Similar to the clamp-derived steady-state insulin sensitivity index ($S_{IP(clamp)}$), the model calculates an insulin sensitivity index, S_I, which is the ratio of two of the fitted parameters (p3/p2). The same group that

invented the FSIGT and minimal model analysis also designed a computer program just for that purpose (Pacini and Bergman 1986). The first minimal model equation describes the rate at which glucose returns to basal level after injection of dextrose:

$$-dG(t)/dt = p_1 [G(t) - G_b] + X(t) G(t)$$

The ratio $dG(t)/dt$ is the change of glucose concentration over time t. The equation states that the rate $-dG(t)/dt$ depends on glucose itself ($G(t)$ is the actual glucose concentration at time point t, G_b is basal glucose), which is proportional to p_1 (see below) and upon $X(t)$, which is designated insulin action in a compartment remote from plasma, multiplied by the glucose concentration (Bergman et al. 1979; Bergman et al. 1989). Parameter p_1 is one of the three fitted parameters of the model and is also called the fractional disappearance rate of glucose at *basal* insulin, which is independent of the insulin response. It equals the parameter of glucose effectiveness, S_G.

The second equation determines the dynamics by which the remote insulin action compartment is filled or emptied:

$$dX(t)/dt = p_3 I(t) - p_2 X(t)$$

$I(t)$ refers to plasma insulin concentration, $X(t)$ equals the effect of insulin accelerating glucose disappearance in a compartment remote from plasma, and p_2 and p_3 are fitted model parameters, estimated from the least-squares fitting of the glucose data. G_b refers to preinjection or basal glucose concentration. Parameters p_2 and p_3 determine the

kinetics of transport into and out of the remote compartment where insulin action is expressed. The equation basically describes that the rate at which the insulin action compartment is filled is proportional to the plasma insulin concentration, I(t), and that the rate at which it empties is proportional to the concentration of insulin in the remote compartment, X(t). As p_3 increases, transport into the remote compartment is more efficient, while a decrease in p_2 represents less efficient emptying of the remote compartment, both ultimately leading to more efficient insulin action to accelerate glucose disappearance (Bergman et al. 1989). Parameters p_2 and p_3 therefore determine the kinetics of transport into and out of the remote insulin compartment. Thus, insulin sensitivity is the ratio of ability to *fill* divided by ability to *empty* the remote compartment:

$$S_I = p_3/p_2$$

S_I is expressed as [$min^{-1}/(\mu U/ml)$] or [$min^{-1}/(nmol/ml)$], representing the fractional disappearance of glucose per insulin concentration unit, which is very similar to $S_{IP(clamp)}$. The index is attractive because it is normalized to body size, allowing for comparison of individuals with varying body composition, size and weight, but also because S_I is independent of ambient plasma glucose and insulin concentrations. The model moreover offers the advantage that insulin sensitivity can be estimated with good precision from a simple intravenous glucose injection. The S_I value for humans is similar to the estimated value in dogs given an equivalent intravenous glucose load (Bergman et al. 1979).

Despite pointing out the pitfalls of the static assessment of insulin action by the clamp technique compared to the dynamic assessment with the Minimal Model analysis earlier, S_I derived from the Minimal Model is very closely correlated to $S_{IP(clamp)}$, and the physiological processes which determine the dynamic and the steady-state relationships between insulin and glucose uptake are, in fact, the same (Bergman et al. 1989). Bergman and Prager, using the tolbutamide-modified FSIGT and the EHC in humans with varying body weights, showed not only a very strong correlation ($r = 0.89$), but that S_I derived from the minimal model analysis of the FSIGT is indeed equivalent to $S_{IP(clamp)}$. In order to compare similar units, conversion factors were used for each model and $S_{IP(clamp)}$ was multiplied by the body surface area, whereas S_I was multiplied by the distribution volume of glucose, V_d (Bergman et al. 1987). Comparison on a subject to subject basis, however, found a mild, but significant tendency for S_I x V_d to exceed $S_{IP(clamp)}$ x surface area. This finding was largely attributed to the fact that the model-derived sensitivity in theory assesses the total effect of insulin on glucose economy. It is therefore defined in terms of the ability of insulin to both augment glucose utilization *and*, at least to some degree, to inhibit hepatic glucose output. Clamp-derived sensitivity, on the other hand, is defined as the action of insulin to augment glucose utilization (R_d) only (Bergman et al. 1987).
Several other studies have validated the FSIGT and minimal model analysis in both dogs and humans (Finegood et al. 1984; Ader et al. 1985; Beard et al. 1986). In dogs, a highly significant correlation ($r = 0.82$) between S_I and $S_{IP(clamp)}$ over a ninefold range of insulin sensitivities has been identified in one of the earlier studies (Finegood et al. 1984).
As already mentioned, one of the model parameters, p_1, is equivalent to glucose effectiveness (S_G) and represents the fractional disappearance rate of glucose at *basal*

insulin concentration or the ability of glucose *per se* to enhance glucose disposal and to suppress endogenous glucose production. As stated earlier, non-insulin mediated glucose uptake (NIMGU) may contribute significantly to the efficient disposition of glucose, especially under conditions of reduced insulin action (Baron et al. 1985; Alzaid et al. 1994) and is therefore an important parameter of glucose tolerance. In addition to being less technically demanding, the FSIGT also offers the advantage of measuring S_I and S_G simultaneously from a single test, whereas two separate clamps must be performed to obtain similar information. Several studies have validated S_G against direct measures of glucose-dependent glucose disappearance, for example derived from the glucose clamp (Ader et al. 1985; Baron et al. 1985). Researchers have argued that the minimal model overestimates the actual importance of glucose effectiveness in subjects with IDDM (Quon et al. 1994) or in dogs where insulin response was blunted by treatment with streptozotocin. This latter study suggested that an overestimation of S_G in dogs with normal insulin secretory response may be due to an artifact of the minimal model method (Finegood and Tzur 1996). Furthermore, some investigators proposed that overestimation of S_G leads to underestimation of S_I in order to maintain a good least squares fit (Quon et al. 1994; Caumo et al. 1996) and that estimation of S_G may not be independent of the insulin secretory response (Finegood and Tzur 1996; Cobelli et al. 1998; Pacini et al. 1998). Other studies found the S_I index was not adversely affected by errors in glucose effectiveness (McDonald et al. 2000). In contrast, studies conducted by Ader et al. and Ward et al. did not find statistically significant differences between S_G estimated by the minimal model and direct measurements, which could be related to the fact that they used a tolbutamide- and insulin- modified FSIGT, respectively, possibly providing different

estimates of S_G (Ader et al. 1985; Ward et al. 1991). Ni et al. proposed that the simplifying assumption of single-compartment glucose kinetics in the minimal model is the reason for overestimation of S_G and the occurrence of S_I values indistinguishable from zero. Comparing both parameters to S_G and S_I derived from clamp studies and a presumably more accurate two-compartment model, they concluded that S_G and S_I from the minimal model are not necessarily equivalent, but that there were strong correlations between $S_{I(MINMOD)}$ and $S_{I(Clamp)}$ ($r = 0.998$) and $S_{G(MINMOD)}$ and $S_{G(Clamp)}$ ($r = 0.996$), respectively. Moreover, they concluded that the one-compartment glucose distribution assumption contributes to a deviation, but not overestimation on average, of $S_{G(MINMOD)}$ in comparison to $S_{G(Clamp)}$ (Ni et al. 1997). In addition, a study comparing S_G from the regular and the insulin-modified FSIGT found a positive correlation ($r = 0.66$) despite a 28% numerical difference between the two estimates (Pacini et al. 1998).

As mentioned earlier, from a physiological standpoint, it is believed that the glucose regulating system in a normal individual can compensate for insulin resistance by increasing plasma insulin concentration. Bergman has described the relationship as a hyperbolic function, representing an inverse relationship between insulin sensitivity and insulin secretion (Bergman et al. 2002; Ahren and Pacini 2004). It describes the generally accepted concept that in order to maintain glucose tolerance, the product of insulin secretion and sensitivity, named disposition index (DI), needs to remain constant, or otherwise hyperglycemia ensues. Individuals with impaired glucose tolerance or type 2 diabetes will therefore have a shift of the curve to the left and downwards (Bergman et al. 2002). As mentioned earlier, pancreatic secretory response is multiphasic, but to assess

insulin secretion, it is the first-phase insulin secretion that is typically measured. The so-called acute insulin response to an intravenous bolus of glucose (AIR_g) is the parameter obtained from the FSIGT. This index represents the average insulin concentration above basal during the first peak (in general from 2-10 minutes) of an IVGTT. The FSIGT offers the advantage of measuring both insulin sensitivity and secretion in one procedure, whereas a hyperglycemic and euglycemic clamp experiment would have to be performed to accurately assess each parameter.

The original or regular FSIGT uses only the patient's endogenous insulin response to estimate insulin sensitivity. However, some publications advocate the so-called modified FSIGT, using tolbutamide, an insulin secretagogue, or exogenously administered regular insulin. Using tolbutamide, one study demonstrated a considerable improvement in accuracy and precision of insulin sensitivity estimation, leading to a reduction of sample size necessary to detect significant differences between groups (Yang et al. 1987). Two other studies showed the modified test to strengthen correlation to the $S_{IP(clamp)}$ considerably (Beard et al. 1986; Bergman et al. 1987). Saad and coworkers showed that insulin sensitivity estimates obtained from insulin-modified and tolbutamide-modified FSIGTs correlate significantly with each other and with the clamp (Saad et al. 1994). The insulin-modified FSIGT is becoming the recommended test to estimate insulin sensitivity in a wide variety of situations (Welch et al. 1990), especially when the endogenous insulin response is lacking, such as in type 1 diabetic patients (Finegood et al. 1990; Ward et al. 1991). In addition, tolbutamide is not easily available anymore and may have extrapancreatic metabolic effects. A study comparing S_I and S_G obtained from the regular

and insulin-modified FSIGT in a group of normal human subjects with various degrees of glucose tolerance showed that S_I was not only equivalent amongst both tests, but also showed a much lower coefficient of variation with the modified FSIGT, leading to a more precise estimate (Pacini et al. 1998). On the other hand, as already mentioned, S_G differed by 28% between the two tests, although a good correlation was still observed, providing further evidence about a possible dependence of S_G on ambient insulin concentrations. By allowing a 20-minute delay between glucose and exogenous tolbutamide or insulin administration, the patient's first peak pancreatic response can still be evaluated and AIR_g calculated. Nevertheless, to assess first- and second-phase insulin secretion and hepatic extraction, some studies still exploit the classic FSIGT.

The reasons for improved estimation of S_I with a stronger correlation to the clamp-based sensitivity with either test has been explained as follows: The overlapping dynamics of glucose disappearance due to glucose itself and due to insulin complicate the distinction between the discrete effects of both parameters on glucose normalization (Yang et al. 1987). Also, the model requires a substantial effect of insulin after an initial glucose mixing period (usually within 8 minutes) to calculate a precise measure of insulin sensitivity. Thus, the modified FSIGTs manipulate the time courses of glucose and insulin and enhance the dynamics of the insulin pattern, providing a better basis for the computer to distinguish insulin effect from the other factors affecting glucose disappearance (Beard et al. 1986; Bergman et al. 1987; Yang et al. 1987; Pacini et al. 1998).

In summary, minimal model analysis yields a measure of insulin sensitivity and glucose effectiveness, which is very similar or even equivalent to the same parameter measured

by the euglycemic glucose clamp. Minimal model analysis, which has the advantages of simplicity, minimal invasiveness, reasonable reflection of non-steady-state glucose kinetics, and cost-effectiveness could in many cases outweigh the structural bias introduced by the model simplification. It may therefore be easier to perform the minimal model method in longitudinal or cross-sectional epidemiologic studies of insulin resistance. Despite the pitfalls in estimation of S_G and S_I, there is a good linear correlation between these same parameters determined by different methods. Therefore, in a study evaluating differences among groups, S_G and S_I can reliably be estimated, regardless of which protocol is used, provided the same type of test is used in both groups.

Chapter II: The effect of hypothyroidism on insulin sensitivity and glucose tolerance

Introduction

Thyroid hormones are integral for normal glucose homeostasis. While hypothyroidism has been suggested to be an uncommon cause of insulin resistance and has been associated with poor glycemic control in diabetic dogs (Ford et al. 1993)., diabetes mellitus was the most common concurrent disease in a retrospective study of hypothyroidism (Dixon et al. 1999). In addition, hypothyroidism was one of the most commonly diagnosed concurrent disorders in dogs with diabetes mellitus (Hess et al. 2000). Experimental studies in dogs with induced hypothyroidism suggest the presence of insulin resistance, as indicated by markedly exaggerated plasma insulin concentrations after oral or intravenous glucose tolerance tests (Renauld et al. 1972; Renauld et al. 1973; Renauld et al. 1974; Renauld et al. 1982). Decreased insulin sensitivity and impaired glucose utilization associated with hypothyroidism have been identified in other species as well (Rochon et al. 2003; Cettour-Rose et al. 2005).

While standard glucose tolerance tests provide a general assessment of glucose tolerance and insulin secretion, more sophisticated evaluation of insulin sensitivity and glucose disposal is provided by other experimental techniques. The frequently-sampled intravenous glucose tolerance test (FSIGT) with minimal model analysis shares good correlation to the gold standard, the euglycemic hyperinsulinemic clamp technique (Bergman et al. 1987). The model not only calculates an index for insulin sensitivity (S_I), but also evaluates other parameters that are considered important variables in maintaining

glucose tolerance, such as insulin secretion and the effect of glucose on its own disappearance. The minimal model therefore provides a more comprehensive view of the glucose regulating system than a simple glucose tolerance test.

Knowledge of the influence of hypothyroidism on glucose metabolism may have important implications in successful glycemic control of the diabetic dog and perhaps in understanding the pathogenesis of diabetes mellitus in some dogs. Thus, the aim of the present study was to evaluate whether hypothyroidism causes insulin resistance and to study the overall effect of hypothyroidism on glucose tolerance in the dog.

Materials and Methods

Animals

Sixteen mixed breed bitches in anestrus, aged 25-39 months and weighing 9-13 kg were utilized for this study. All dogs were determined to be healthy based on routine physical examinations, complete blood count, serum chemistry, heartworm antigen test, and zinc sulfate floatation. They were housed individually under controlled kennel conditions at the Virginia Maryland Regional College of Veterinary Medicine and fed a commercially available standard diet [a]. Hypothyroidism was induced in eight randomly selected dogs by administering 1 mCi/kg ^{131}I, 257 to 404 days prior to the start of the study, while the remaining 8 dogs were euthyroid controls. Hypothyroidism was confirmed by documenting a serum thyroxine (T4) concentration < 5 nmol/L before and 4 hours after IV administration of 50 µg human recombinant thyroid stimulating hormone (TSH) [b] 9 weeks and 38-45 weeks after ^{131}I. The euthyroid dogs had post-TSH serum T4 concentrations > 35 nmol/L. All animal experiments were performed in the morning after

a 12-hour fasting period. All experiments were performed in conscious animals and experimental procedures were approved by the Virginia Tech Animal Care and Use Committee.

Insulin-modified frequently sampled intravenous glucose tolerance test

Insulin-modified FSIGT protocols were performed as described previously (Tobin and Finegood 1993) with slight modifications. On the day of the experiment, an 18 G [c] and a 20 G [d] indwelling catheter were placed in the jugular and cephalic vein, respectively. Animals were rested quietly for 30 minutes in a cage and 5 minutes on the table before starting experiments. Two milliliters of venous blood were obtained through the jugular catheter, which was flushed with 1 ml of heparinized saline (5 U/ml) before and after each sampling. The total dose of heparin did not exceed 600 U/animal. Four blood samples were drawn at –20, -10, -5 and –1 min for fasting (baseline) plasma insulin and glucose determinations. Exogenous glucose (0.3 g/kg) as 25% dextrose in 0.45% saline [e] was administered intravenously through the cephalic catheter over one minute at time 0. Human recombinant regular insulin [f], at a dose of 0.03 U/kg, was administered through the cephalic catheter over 10 seconds at t = 20 minutes. On the morning of each experiment, insulin was diluted in 0.9% sodium chloride to a final concentration of 1U/ml. In addition to the 4 baseline samples, twenty-six additional samples were drawn at 2, 3, 4, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 100, 120, 140, 160 and 180 minutes. Blood was immediately placed in chilled tubes coated with 5 mg sodium fluoride and 4 mg potassium oxalate [d] and stored on ice. Within 30 min of

collection, samples were centrifuged at 2500 x g at 4°C for 12 min. Plasma was separated and was frozen in 0.2 – 0.4 ml aliquots at –70°C for later analysis.

Minimal model analysis

Fasting or basal plasma glucose and insulin concentrations were calculated by averaging the values of the initial four samples obtained before glucose injections were given. Insulin sensitivity (S_I) and glucose effectiveness (S_G) were calculated by using the computer program MINMOD, as described in detail elsewhere (Pacini and Bergman 1986). The S_I index (in [min^{-1} per µU/ml]), defined as the fractional glucose uptake rate per unit plasma insulin, represents the ability of insulin to enhance glucose uptake from plasma and to inhibit glucose release into plasma. Glucose effectiveness, S_G ([min^{-1}]), reflects the effect of glucose to enhance its own disappearance from plasma independently of a rise in plasma insulin above basal level.

In addition, the acute insulin response to glucose injection (AIR_g) was calculated as the area under the curve of the insulin concentrations from 0 to 19 minutes after glucose administration. This index represents the average insulin concentration above basal during the first peak of an intravenous glucose tolerance test and is a measure of endogenous insulin secretion. The disposition index (DI) was calculated as the product of AIR_g and S_I. It represents a measure of overall glucose tolerance and an index of ß-cell function that describes the relation between insulin secretion and insulin sensitivity (Mari et al. 2005).

Analysis of glucose and insulin

Plasma glucose concentrations were determined in duplicate with a chemistry analyzer using the hexokinase enzyme method [g].

For insulin determination, a competitive binding, double antibody radioimmunoassay [h] was used according to the manufacturer's instructions. All samples, controls and standards were assayed in duplicate and counted with an automated gamma counter [i].

The insulin radioimmunoassay was validated for canine plasma collected in tubes containing sodium fluoride and potassium oxalate by determining sensitivity, precision, and linearity of dilution. Sensitivity was determined by calculating the point of 95% of total binding on the standard curve. Interassay precision was determined by calculating the coefficient of variation (CV) of 3 pools of canine plasma containing low, medium and high concentrations of insulin in 7 different assays. Intra-assay precision was calculated as the coefficient of variation from the mean of 10 replicates of each of the three plasma pools measured in one assay. Linearity of dilution was evaluated by measuring insulin concentrations on a plasma sample with expected elevated endogenous insulin concentrations and on a sample obtained after exogenous insulin administration. Each sample was diluted with the 0 μIU/ml insulin standard supplied with the kit at 1:1, 1:2, 1.4 and 1:8 dilution.

Statistical analysis

Experimental data are reported as means +/- SD unless stated otherwise. Data was analyzed for normality and equal variances. Statistical comparison of means between

hypothyroid and control group was performed using the pooled-variance t-test for equal variances or Welch-Satterthwaite t-test for unequal variances. *P*-values < 0.05 were considered to be significant. By using correlation and simple regression analyses, data of each group was analyzed for relationships between variables derived from the minimal model analysis and body weight. In addition to the mean, 95% confidence intervals of the correlation coefficients are reported also. Statistical analyses were performed with a proprietary statistical program [j].

Results

Assay Validation

Sensitivity of the insulin assay was 1µU/ml. The interassay CV for low (4 µU/ml), medium (19.1 µU/ml) and high (40 µU/ml) insulin concentrations was 12.3%, 11.1% and 9%, respectively. The intraassay CV for low (4 µU/ml), medium (20.6 µU/ml) and high (46.3 µU/ml) insulin concentrations was 18.4%, 6% and 5.9%.

Percentage recovery after serial dilutions was 97%, 92%, 106% and 118% for the sample with endogenous insulin with an initial concentration of 54 µU/ml. For the sample containing both canine and human recombinant insulin, percentage recovery could not be calculated for the 1:1 dilution, as sample volume was insufficient to measure duplicates. Otherwise, recovery rates, compared to the 1:1 dilution with a concentration of 106 µU/ml were 92%, 82% and 90%, respectively, at 1:2, 1:4 and 1:8 dilutions.

Insulin-modified FSIGT and minimal model analysis

One dog in the control group was excluded from analysis due to an exaggerated response to the exogenously administered insulin, which caused prolonged hypoglycemia below 50 mg/dl between 25 and 40 minutes.

Prior to induction of hypothyroidism, average body weight of each group was similar (control group 9.7 +/- 1.18 kg, hypothyroid group 9.8 kg +/- 0.77 kg). At the time of the experiments, hypothyroid dogs weighed more ($P = 0.0064$) than euthyroid dogs (11.9 +/- 0.59 kg and 10.4 kg +/- 1.23, respectively).

Average insulin and glucose concentrations are shown in Figures 1 and 2. Basal insulin concentrations were higher ($P = 0.019$) (8.2 µIU/ml +/- 3.23 versus 4.2 µIU/ml +/- 2.32), whereas mean glucose concentrations were lower ($P = 0.0079$) in the hypothyroid group (90.1 mg/dl +/- 4.4 versus 96.4 mg/dl +/- 3.16). As expected, a first-phase endogenous insulin response was observed (Figure 1), corresponding to the acute insulin response to glucose injection (AIR_g), followed by a second peak that was caused by exogenous administration of human recombinant regular insulin. The AIR_g was higher ($P = 0.01$) in the hypothyroid dogs (459 µU/ml +/- 258) compared to euthyroid controls (159 µU/ml +/- 55.9).

Mean S_I for hypothyroid dogs was lower ($P < 0.0001$) than that of the euthyroid group (4.9×10^{-4} min^{-1}/µU/ml +/- 1.2 and 23.6×10^{-4} min^{-1}/µU/ml +/- 5.31, respectively). In contrast, glucose effectiveness, S_G, was similar between control and hypothyroid groups (0.043 min^{-1} +/- 0.0088 versus 0.043 min^{-1} +/- 0.0108) ($P = 0.977$).

The disposition index (DI) was numerically decreased in the hypothyroid group (2244 min^{-1} +/- 1386), but compared with the euthyroid group (3376 min^{-1} +/- 1616), the difference was not significant (*P* = 0.0696).

No important association was found between parameters of the model analysis and body weight (Table 1).

Discussion

The results of this study indicate that hypothyroidism caused substantial insulin resistance in our study dogs, as evidenced by an almost fivefold decrease in the S_I index. The reduction in insulin sensitivity noted in this study is slightly greater than that found in obese dogs using the euglycemic hyperinsulinemic clamp (EHC) (Ellmerer et al. 2006). Moreover, by looking at other parameters from the minimal model analysis, our results show that hypothyroid dogs are able to maintain glucose tolerance by a compensatory increase in ß-cell response and by normal glucose effectiveness. These findings expand on previous work in dogs that demonstrated glucose intolerance, but only inferred insulin resistance. In that series of studies (Renauld et al. 1972; Renauld et al. 1973; Renauld et al. 1974), Renauld and coworkers consistently demonstrated exaggerated insulin responses during glucose tolerance tests in hypothyroid dogs, which is consistent with our observations. Glucose concentrations and disappearance rates, used to assess glucose tolerance, were either unchanged (Renauld et al. 1972; Renauld et al. 1973) or showed a delayed return to basal levels in hypothyroid dogs (Renauld et al. 1973; Renauld et al. 1974). While these studies suggest a causal relationship between hypothyroidism and insulin resistance, the minimal model analysis of the FSIGT utilized in the present study allowed a more detailed assessment of insulin sensitivity and glucose

tolerance. Our results are in agreement with human studies (Arner et al. 1984; Pedersen et al. 1988; Rochon et al. 2003; Stanicka et al. 2005) and rodents (Dimitriadis et al. 1997; Cettour-Rose et al. 2005) showing that hypothyroidism causes insulin resistance and negatively affects overall glucose utilization.

To better understand the mechanisms underlying glucose intolerance, we utilized the FSIGT with minimal model analysis, as indices of insulin sensitivity derived from this technique are highly correlated with those obtained using the EHC, a technique that is considered the gold standard for estimation of insulin sensitivity (Beard et al. 1986; Bergman et al. 1987). The FSIGT was developed to allow a dynamic approach to the assessment of insulin sensitivity and other factors that affect glucose metabolism (Bergman et al. 1979; Toffolo et al. 1980). The minimal model of glucose and insulin kinetics represents the least complex mathematical model that relates to the effect of glucose itself (glucose effectiveness, S_G) and the effect of insulin (insulin sensitivity, S_I) to promote glucose uptake and inhibit its production and release by the liver (hepatic glucose output, HGO). A computer program, using a nonlinear least squares estimation method, searches for the best fit of insulin and glucose data, yielding parameters of S_I, S_G and first phase pancreatic responsivity (Pacini and Bergman 1986).

A decreased S_I in hypothyroid dogs could result from a decrease in insulin action on glucose disposal at insulin-sensitive sites such as skeletal muscle and adipose tissue or from a diminished effect of insulin on endogenous glucose production by suppression of hepatic gluconeogenesis and glycogenolysis. Although S_I, as calculated in this study,

does not provide information about glucose metabolism in specific tissues or what the underlying mechanism for insulin resistance is, reduced glucose transport and disposal (Arner et al. 1984; Pedersen et al. 1988; Dimitriadis et al. 1997; Cettour-Rose et al. 2005) as well as decreased rates of glycolysis (Dimitriadis et al. 1989; Dimitriadis et al. 1997) at peripheral tissue sites has been found in skeletal muscle and adipose tissue of hypothyroid rats and humans. Reduction of the insulin-sensitive glucose transporters (GLUT 4) has been found in skeletal muscle from hypothyroid rats and is thought to account in part for the decreased glucose transport (Weinstein et al. 1991). Conversely, hepatic gluconeogenesis (Okajima and Ui 1979; Comte et al. 1990) and rates of glycogenolysis and hepatic glucose release in response to insulin (Goldblatt 1936) are diminished in hypothyroid rats and rabbits, respectively. These studies indicate that hypothyroidism does not negatively affect hepatic insulin sensitivity and HGO and that insulin resistance at peripheral sites may be, at least in part, counterbalanced by a decreased glucose output by the liver, thereby maintaining euglycemia. Reduced gluconeogenesis and glycogenolysis could account for the finding of lower, albeit normal fasting glucose levels in the hypothyroid dogs of the present study.

The finding of lower basal glucose levels, in this study, moreover illustrate that mechanisms other than increased glucose concentrations must be the stimulus for the increased insulin levels. Reduced insulin clearance has been documented in dogs with obesity-induced insulin resistance (Mittelman et al. 2002), thereby providing a higher proportion of secreted insulin to the periphery. This in turn may be applicable to hypothyroidism-induced insulin resistance, as increased insulin clearance and decreased insulin half-life have been documented in hyperthyroidism (Dimitriadis et al. 1985;

Randin et al. 1986). Further studies are needed in hypothyroid dogs to assess the differential roles of insulin secretion, clearance and hepatic extraction on plasma insulin concentrations in order to determine their relative contributions to the increased plasma insulin concentrations found in our study. In addition, FSIGT studies including [^{3}H]-labeled glucose would allow identification of the contribution of glucose utilization and endogenous glucose production on insulin resistance (Cobelli et al. 1986). Furthermore, evaluation of skeletal muscle and adipose tissue biopsies of hypothyroid dogs for GLUT 4 could help elucidate the pathogenesis of insulin resistance.

Other potential mechanisms of insulin resistance due to hypothyroidism have not been explored in detail. Insulin binding to receptors and receptor number have been shown to be either increased or decreased (Arner et al. 1984; Pedersen et al. 1988). In addition to the direct effects of hypothyroidism on insulin sensitivity, it may alter other factors important in glucose homeostasis. Hypothyroidism in dogs is often associated with obesity, which can cause insulin resistance (Ellmerer et al. 2006). More specifically, insulin resistance has been linked to central or abdominal obesity (Karter et al. 2005; Bergman et al. 2007) and abnormal concentrations of adipocytokines (Bastard et al. 2006; Kong et al. 2006). A recent study in hypothyroid rats found changes in the glucose-fatty acid cycle causing decreased glucose disposal to be related to lowered plasma leptin and increased resistin levels (Cettour-Rose et al. 2005). In our study, hypothyroid dogs weighed significantly more than the control dogs despite being fed the similar amounts of a diet that maintained body weight, prior to induction of hypothyroidism. It is possible that changes in body composition caused by hypothyroidism could contribute to the decreased insulin sensitivity. While no significant association was found between body

weight and S_I in the present study, a more detailed analysis looking at body fat amount and distribution as well as measurement of adipocytokines, such as leptin or adiponectin may add further information to the potential role of adipose tissue on glucose metabolism.

Increased amounts of counter-regulatory hormones to insulin may also play a role in the pathogenesis of insulin resistance. Despite the lack of correlation between markers of insulin sensitivity and cortisol, glucagon, epinephrine and growth hormone in a group of hypothyroid women with insulin resistance, those hormones were markedly increased before hormone replacement therapy (Stanicka et al. 2005). Elevated growth hormone (GH) levels have recently been found in hypothyroid dogs (Lee et al. 2001) and may contribute to insulin resistance, possibly through insulin-like growth factor I (IGF-I) mediation.

Glucose effectiveness (S_G), defined as the fractional disappearance rate of glucose at basal insulin concentrations, can be viewed as the ability of glucose per se to enhance its own uptake and, to a lesser degree, to suppress endogenous glucose production by the liver, independent of insulin (Ader et al. 1997). The effect of S_G has been shown in dogs to be at least as important as the dynamic insulin response to the normalization of glucose and thus glucose tolerance (Ader et al. 1985; Ader et al. 1997). In humans with non-insulin dependent diabetes mellitus (NIDDM), glucose effectiveness was unaffected despite profound insulin resistance (Baron et al. 1985; Alzaid et al. 1994), which is therefore believed to resume a primary role in overall glucose disposal. In our study, values obtained for S_G were virtually identical between groups, indicating that

hypothyroidism had no effect on this parameter. Because insulin resistance reduces glucose disposal at insulin sensitive sites, glucose effectiveness in hypothyroid dogs may therefore be of relatively greater importance in hypothyroid than euthyroid dogs.

The AIR_g was nearly threefold higher in the hypothyroid dogs compared to control dogs in our study. It is considered another mechanism of compensation for insulin resistance. The magnitude of change in secretion also explains why the disposition index (DI), derived from insulin secretion and sensitivity (AIR_g x S_I) was not significantly different between groups. This indicates that the compensatory insulin secretory response was sufficient to sustain normal glucose tolerance. The DI reflects a fundamental concept in the understanding of ß-cell function and indicates that change in one of the variables in a healthy individual is mirrored by a reciprocal change in the other variable, otherwise hyperglycemia develops (Ahren and Pacini 2004). Applying this concept to the results of this study, it suggests that hypothyroid dogs have sufficient ß-cell function to remain glucose tolerant and euglycemic. Furthermore, it illustrates why hypothyroid dogs with concurrent diabetes may be difficult to regulate, as they are unable to compensate for insulin resistance by secretion of insulin necessary to overcome this defect. Our results are comparable with observations made in dogs with spontaneous hypothyroidism (Ford et al. 1993), in which glycemic control of diabetic dogs was thought to be difficult due to the concurrent presence of hypothyroidism. Importantly, as glycemic control improved with treatment of hypothyroidism, insulin resistance may have been reversible, similar to what has been found in humans (Rochon et al. 2003; Stanicka et al. 2005). Additional studies in dogs with sufficient case numbers are desirable to strengthen this evidence.

However, hypothyroid dogs may not remain glucose tolerant as the disease progresses, and it is tempting to speculate whether they are more prone to develop diabetes mellitus due to earlier exhaustion of pancreatic ß- cells from either chronic over-stimulation or concurrent presence of pancreatic disease. This has been shown in humans with type 2 diabetes, where impaired ß-cell secretion occurs concurrent with insulin resistance, even before the onset of overt hyperglycemia (Weyer et al. 1999). Longitudinal evaluation in dogs with diet-induced obesity and subsequent development of insulin resistance found the AIR_g to be markedly increased initially, followed by a decline after a few weeks that lead to a 50% decrease of the DI by the end of the study (Mittelman et al. 2000). Although a similar study has not been performed in hypothyroid dogs, diabetes mellitus was present in 10% of hypothyroid dogs in one retrospective study (Dixon et al. 1999) and hypothyroidism was one of the most common concurrent diseases in dogs with diabetes mellitus (Hess et al. 2000). In addition, the risk of developing acute pancreatitis may be increased by hypothyroidism (Hess et al. 1999), which could reduce ß-islet cell function and reduce compensatory insulin secretion necessary for maintaining euglycemia.

In conclusion, hypothyroid dogs in the present study were markedly more insulin resistant than euthyroid controls. Moreover, the FSIGT and minimal model analysis provided valuable information about the effect of hypothyroidism on overall glucose disposal, demonstrating normal glucose tolerance through increased insulin secretion and unchanged glucose effectiveness. Knowledge of the dog's thyroid status therefore could have important implications in the successful management of diabetes mellitus in dogs.

Footnotes

[a] Hill's Science Diet Adult dry kibble, Topeka, KS

[b] Thyrogen, Gezyme Corp., Framingham, MA

[c] Venocath, Abbott Laboratories, Abbott Park, IL

[d] Becton Dickinson, Franklin Lakes, NJ

[e] Vedco Inc., St. Joseph, MO

[f] Humulin R, Eli Lilly, Lake Forest, IL

[g] Olympus AU 400 Chemistry Analyzer and OSR6121 Glucose Reagent, Olympus America Inc., Melville, NY

[h] DSL-1600 Insulin radioimmunoassay, Diagnostic Systems Laboratories, Inc., Webster, TX

[i] Packard Cobra II Gamma Counter, Packard, Meriden, CT

[j] SAS 9.1.3, SAS Institute, Cary, NC

Conclusion

In conclusion, this study provided new valuable information about the influence of hypothyroidism on factors that affect glucose tolerance. The presence of marked insulin resistance was compensated by a nearly threefold increase in insulin secretion and normal glucose effectiveness, thereby maintaining glucose tolerance and euglycemia. Our results not only place emphasis on the importance of detecting hypothyroidism for successful management of diabetes mellitus, but also point out that hypothyroidism may be a potential risk factor for the development of ß-cell exhaustion.

Further studies evaluating the levels of counterregulatory hormones to insulin, the influence of changes in adipose tissue and adipocytokines as well as GLUT 4 concentrations in skeletal muscle and adipose tissue sites may provide insights into the underlying mechanisms leading to insulin resistance. Furthermore, the assessment of minimal model parameters in dogs with naturally occurring hypothyroidism before and after hormone replacement therapy, with or without concurrent diabetes mellitus, would be of interest. This would extend the implications of our study to a more general canine population and would evaluate whether changes in insulin sensitivity are reversible with hormone replacement therapy.

Bibliography

Ader, M. and R. Bergman (1987). Insulin sensitivity in the intact organism. Bailliere's Clinics in Endocrinology and Metabolism. Techniques for Metabolic Investigation in Man. K. G. Alberti, P. D. Home and R. Taylor. London. **1, Part 1:** 879-910.

Ader, M., T. C. Ni, et al. (1997). "Glucose effectiveness assessed under dynamic and steady state conditions. Comparability of uptake versus production components." J Clin Invest **99**(6): 1187-99.

Ader, M., G. Pacini, et al. (1985). "Importance of glucose per se to intravenous glucose tolerance. Comparison of the minimal-model prediction with direct measurements." Diabetes **34**(11): 1092-103.

Ahren, B. and G. Pacini (2004). "Importance of quantifying insulin secretion in relation to insulin sensitivity to accurately assess beta cell function in clinical studies." Eur J Endocrinol **150**(2): 97-104.

Alzaid, A. A., S. F. Dinneen, et al. (1994). "Assessment of insulin action and glucose effectiveness in diabetic and nondiabetic humans." J Clin Invest **94**(6): 2341-8.

American Diabetes Association, A. D. (1998). "Consensus Development Conference on Insulin Resistance. 5-6 November 1997. American Diabetes Association." Diabetes Care **21**(2): 310-4.

Arner, P., J. Bolinder, et al. (1984). "Influence of thyroid hormone level on insulin action in human adipose tissue." Diabetes **33**(4): 369-75.

Baron, A. D., O. G. Kolterman, et al. (1985). "Rates of noninsulin-mediated glucose uptake are elevated in type II diabetic subjects." J Clin Invest **76**(5): 1782-8.

Bastard, J. P., M. Maachi, et al. (2006). "Recent advances in the relationship between obesity, inflammation, and insulin resistance." Eur Cytokine Netw **17**(1): 4-12.

Beard, J. C., R. N. Bergman, et al. (1986). "The insulin sensitivity index in nondiabetic man. Correlation between clamp-derived and IVGTT-derived values." Diabetes **35**(3): 362-9.

Bell, G. I., T. Kayano, et al. (1990). "Molecular biology of mammalian glucose transporters." Diabetes Care **13**(3): 198-208.

Bergman, R. N., M. Ader, et al. (2002). "Accurate assessment of beta-cell function: the hyperbolic correction." Diabetes **51 Suppl 1**: S212-20.

Bergman, R. N., D. T. Finegood, et al. (1985). "Assessment of insulin sensitivity in vivo." Endocr Rev **6**(1): 45-86.

Bergman, R. N., I. D. Hope, et al. (1989). "Assessment of insulin sensitivity in vivo: a critical review." Diabetes Metab Rev **5**(5): 411-29.

Bergman, R. N., Y. Z. Ider, et al. (1979). "Quantitative estimation of insulin sensitivity." Am J Physiol **236**(6): E667-77.

Bergman, R. N., S. P. Kim, et al. (2007). "Abdominal obesity: role in the pathophysiology of metabolic disease and cardiovascular risk." Am J Med **120**(2 Suppl 1): S3-8; discussion S29-32.

Bergman, R. N., L. S. Phillips, et al. (1981). "Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-

cell glucose sensitivity from the response to intravenous glucose." J Clin Invest **68**(6): 1456-67.

Bergman, R. N., R. Prager, et al. (1987). "Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp." J Clin Invest **79**(3): 790-800.

Bratanova-Tochkova, T. K., H. Cheng, et al. (2002). "Triggering and augmentation mechanisms, granule pools, and biphasic insulin secretion." Diabetes **51 Suppl 1**: S83-90.

Bratusch-Marrain, P. R., S. Gasic, et al. (1984). "Triiodothyronine increases splanchnic release and peripheral uptake of glucose in healthy humans." Am J Physiol **247**(5 Pt 1): E681-7.

Bratusch-Marrain, P. R., M. Komjati, et al. (1985). "Glucose metabolism in noninsulin-dependent diabetic patients with experimental hyperthyroidism." J Clin Endocrinol Metab **60**(6): 1063-8.

Bruchim, Y., E. Klement, et al. (2006). "Heat stroke in dogs: A retrospective study of 54 cases (1999-2004) and analysis of risk factors for death." J Vet Intern Med **20**(1): 38-46.

Burchell, A. (1998). "A re-evaluation of GLUT 7." Biochem J **331 (Pt 3)**: 973.

Butler, A. A. and D. L. Roith (2001). "Control of Growth by the Somatotropic Axis: Growth Hormone and the Insulin-Like Growth Factors Have Related and Independent Roles." Annual Review of Physiology **63**(1): 141-164.

Carroll, P. V., E. R. Christ the members of Growth Hormone Research Society Scientific Committee, et al. (1998). "Growth Hormone Deficiency in Adulthood and the

Effects of Growth Hormone Replacement: A Review." J Clin Endocrinol Metab **83**(2): 382-395.

Caumo, A., P. Vicini, et al. (1996). "Is the minimal model too minimal?" Diabetologia **39**(8): 997-1000.

Cettour-Rose, P., C. Theander-Carrillo, et al. (2005). "Hypothyroidism in rats decreases peripheral glucose utilisation, a defect partially corrected by central leptin infusion." Diabetologia **48**(4): 624-33.

Clausen, N., P. E. Lins, et al. (1986). "Counterregulation of insulin-induced hypoglycaemia in primary hypothyroidism." Acta Endocrinol (Copenh) **111**(4): 516-21.

Cobelli, C., F. Bettini, et al. (1998). "Overestimation of minimal model glucose effectiveness in presence of insulin response is due to undermodeling." Am J Physiol Endocrinol Metab **275**(6): E1031-1036.

Cobelli, C., G. Pacini, et al. (1986). "Estimation of insulin sensitivity and glucose clearance from minimal model: new insights from labeled IVGTT." Am J Physiol **250**(5 Pt 1): E591-8.

Comte, B., H. Vidal, et al. (1990). "Influence of thyroid hormones on gluconeogenesis from glycerol in rat hepatocytes: a dose-response study." Metabolism **39**(3): 259-63.

Cowan, J. S., P. Gaul, et al. (1984). "Secretory bursts of growth hormone secretion in the dog may be initiated by somatostatin withdrawal." Can J Physiol Pharmacol **62**(2): 199-207.

Crispin, S. M. and K. C. Barnett (1978). "Arcus lipoides corneae secondary to hypothyroidism in the Alsatian." J Small Anim Pract **19**(2): 127-42.

DeFronzo, R. A., V. Soman, et al. (1978). "Insulin binding to monocytes and insulin action in human obesity, starvation, and refeeding." J Clin Invest **62**(1): 204-13.

DeFronzo, R. A., J. D. Tobin, et al. (1979). "Glucose clamp technique: a method for quantifying insulin secretion and resistance." Am J Physiol **237**(3): E214-23.

DeGroot, L. J., J. L. Jameson, et al. (2001). Endocrinology. Philadelphia, W. B. Saunders Co.

Dessein, P. H., B. I. Joffe, et al. (2004). "Subclinical hypothyroidism is associated with insulin resistance in rheumatoid arthritis." Thyroid **14**(6): 443-6.

Dimitriadis, G., B. Baker, et al. (1985). "Effect of thyroid hormone excess on action, secretion, and metabolism of insulin in humans." Am J Physiol **248**(5 Pt 1): E593-601.

Dimitriadis, G., M. Parry-Billings, et al. (1997). "The effects of insulin on transport and metabolism of glucose in skeletal muscle from hyperthyroid and hypothyroid rats." Eur J Clin Invest **27**(6): 475-83.

Dimitriadis, G. D., B. Leighton, et al. (1989). "Effects of hypothyroidism on the sensitivity of glycolysis and glycogen synthesis to insulin in the soleus muscle of the rat." Biochem J **257**(2): 369-73.

Dixon, R. M., S. W. Reid, et al. (1999). "Epidemiological, clinical, haematological and biochemical characteristics of canine hypothyroidism." Vet Rec **145**(17): 481-7.

Eden, S. (1979). "Age- and sex-related differences in episodic growth hormone secretion in the rat." Endocrinology **105**(2): 555-60.

Elahi, D. (1996). "In praise of the hyperglycemic clamp. A method for assessment of beta-cell sensitivity and insulin resistance." Diabetes Care **19**(3): 278-86.

Ellmerer, M., M. Hamilton-Wessler, et al. (2006). "Reduced access to insulin-sensitive tissues in dogs with obesity secondary to increased fat intake." Diabetes **55**(6): 1769-75.

Feng, X., Y. Jiang, et al. (2000). "Thyroid hormone regulation of hepatic genes in vivo detected by complementary DNA microarray." Mol Endocrinol **14**(7): 947-55.

Ferrannini, E., A. Natali, et al. (1997). "Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR)." J Clin Invest **100**(5): 1166-73.

Finegood, D. T., I. M. Hramiak, et al. (1990). "A modified protocol for estimation of insulin sensitivity with the minimal model of glucose kinetics in patients with insulin-dependent diabetes." J Clin Endocrinol Metab **70**(6): 1538-49.

Finegood, D. T., G. Pacini, et al. (1984). "The insulin sensitivity index. Correlation in dogs between values determined from the intravenous glucose tolerance test and the euglycemic glucose clamp." Diabetes **33**(4): 362-8.

Finegood, D. T. and D. Tzur (1996). "Reduced glucose effectiveness associated with reduced insulin release: an artifact of the minimal-model method." Am J Physiol **271**(3 Pt 1): E485-95.

Ford, S. L., R. W. Nelson, et al. (1993). "Insulin resistance in three dogs with hypothyroidism and diabetes mellitus." J Am Vet Med Assoc **202**(9): 1478-80.

French, M. B., P. Vaitkus, et al. (1987). "Secretory pattern of canine growth hormone." Am J Physiol **252**(2 Pt 1): E268-72.

Galante, P., L. Mosthaf, et al. (1995). "Acute hyperglycemia provides an insulin-independent inducer for GLUT4 translocation in C2C12 myotubes and rat skeletal muscle." Diabetes **44**(6): 646-51.

Gastaldelli, A., K. Cusi, et al. (2007). "Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects." Gastroenterology **133**(2): 496-506.

Gobello, C., Y. A. Corrada, et al. (2002). "Secretory patterns of growth hormone in dogs: circannual, circadian, and ultradian rhythms." Can J Vet Res **66**(2): 108-11.

Goldblatt, M. W. (1936). "Insulin and the thyroidectomized rabbit." J Physiol **86**(1): 46-60.

Gottesman, I., L. Mandarino, et al. (1984). "Use of glucose uptake and glucose clearance for the evaluation of insulin action in vivo." Diabetes **33**(2): 184-91.

Gould, G. W. and G. D. Holman (1993). "The glucose transporter family: structure, function and tissue-specific expression." Biochem J **295 (Pt 2)**: 329-41.

Gourmelen, M., Y. Le Bouc, et al. (1984). "Serum levels of insulin-like growth factor (IGF) and IGF binding protein in constitutionally tall children and adolescents." J Clin Endocrinol Metab **59**(6): 1197-203.

Guyton, A. C. and J. E. Hall (2000). Textbook of Medical Physiology, W.B. Saunders Company.

Hartman, M. L., P. E. Clayton, et al. (1993). "A low dose euglycemic infusion of recombinant human insulin-like growth factor I rapidly suppresses fasting-enhanced pulsatile growth hormone secretion in humans." J Clin Invest **91**(6): 2453-62.

Henriksen, E. J. (2002). "Invited review: Effects of acute exercise and exercise training on insulin resistance." J Appl Physiol **93**(2): 788-96.

Hespel, P., L. Vergauwen, et al. (1995). "Important role of insulin and flow in stimulating glucose uptake in contracting skeletal muscle." Diabetes **44**(2): 210-5.

Hess, R. S., P. H. Kass, et al. (1999). "Evaluation of risk factors for fatal acute pancreatitis in dogs." J Am Vet Med Assoc **214**(1): 46-51.

Hess, R. S., H. M. Saunders, et al. (2000). "Concurrent disorders in dogs with diabetes mellitus: 221 cases (1993-1998)." J Am Vet Med Assoc **217**(8): 1166-73.

Hew, F. L., M. Koschmann, et al. (1996). "Insulin resistance in growth hormone-deficient adults: defects in glucose utilization and glycogen synthase activity." J Clin Endocrinol Metab **81**(2): 555-64.

Higgins, M. A., J. H. Rossmeisl, Jr., et al. (2006). "Hypothyroid-associated central vestibular disease in 10 dogs: 1999-2005." J Vet Intern Med **20**(6): 1363-9.

Hilsted, J., E. Richter, et al. (1987). "Metabolic and cardiovascular responses to epinephrine in diabetic autonomic neuropathy." N Engl J Med **317**(7): 421-6.

Holland, W. L., T. A. Knotts, et al. (2007). "Lipid mediators of insulin resistance." Nutr Rev **65**(6 Pt 2): S39-46.

Jaggy, A., J. E. Oliver, et al. (1994). "Neurological manifestations of hypothyroidism: a retrospective study of 29 dogs." J Vet Intern Med **8**(5): 328-36.

Jansson, J. O., S. Eden, et al. (1985). "Sexual dimorphism in the control of growth hormone secretion." Endocr Rev **6**(2): 128-50.

Johansson, J. O., J. Fowelin, et al. (1995). "Growth hormone-deficient adults are insulin-resistant." Metabolism **44**(9): 1126-9.

Kaneko, J. J., J. W. Harvey, et al. (1997). Clinical Biochemistry of Domestic Animals, Academic Press.

Karlander, S. G., A. Khan, et al. (1989). "Glucose turnover in hyperthyroid patients with normal glucose tolerance." J Clin Endocrinol Metab **68**(4): 780-6.

Karter, A. J., R. B. D'Agostino, Jr., et al. (2005). "Abdominal obesity predicts declining insulin sensitivity in non-obese normoglycaemics: the Insulin Resistance Atherosclerosis Study (IRAS)." Diabetes Obes Metab **7**(3): 230-8.

Kern, T. J. and R. C. Riis (1980). "Ocular manifestations of secondary hyperlipidemia associated with hypothyroidism and uveitis in a dog." J Am Anim Hosp Assoc **16**: 907-14.

Kim, S. P., M. Ellmerer, et al. (2003). "Primacy of hepatic insulin resistance in the development of the metabolic syndrome induced by an isocaloric moderate-fat diet in the dog." Diabetes **52**(10): 2453-60.

Knowler, W. C., D. J. Pettitt, et al. (1981). "Diabetes incidence in Pima indians: contributions of obesity and parental diabetes." Am J Epidemiol **113**(2): 144-56.

Kong, A. P., N. N. Chan, et al. (2006). "The role of adipocytokines and neurohormonal dysregulation in metabolic syndrome." Curr Diabetes Rev **2**(4): 397-407.

Krinsley, J. S. and A. Grover (2007). "Severe hypoglycemia in critically ill patients: Risk factors and outcomes*." Crit Care Med.

Kruszynska, Y. T., P. D. Home, et al. (1986). "In vivo regulation of liver and skeletal muscle glycogen synthase activity by glucose and insulin." Diabetes **35**(6): 662-7.

Le Roith, D., C. Bondy, et al. (2001). "The somatomedin hypothesis: 2001." Endocr Rev **22**(1): 53-74.

Lee, W. M., M. Diaz-Espineira, et al. (2001). "Primary hypothyroidism in dogs is associated with elevated GH release." J Endocrinol **168**(1): 59-66.

Manolescu, A. R., K. Witkowska, et al. (2007). "Facilitated Hexose Transporters: New Perspectives on Form and Function." Physiology **22**(4): 234-240.

Mari, A., B. Ahren, et al. (2005). "Assessment of insulin secretion in relation to insulin resistance." Curr Opin Clin Nutr Metab Care **8**(5): 529-33.

Matthaei, S., M. Stumvoll, et al. (2000). "Pathophysiology and pharmacological treatment of insulin resistance." Endocr Rev **21**(6): 585-618.

Mattheeuws, D., R. Rottiers, et al. (1984). "Diabetes mellitus in dogs: relationship of obesity to glucose tolerance and insulin response." Am J Vet Res **45**(1): 98-103.

McCulloch, A. J., P. D. Home, et al. (1983). "Insulin sensitivity in hyperthyroidism: measurement by the glucose clamp technique." Clin Endocrinol (Oxf) **18**(4): 327-33.

McDonald, C., A. Dunaif, et al. (2000). "Minimal-model estimates of insulin sensitivity are insensitive to errors in glucose effectiveness." J Clin Endocrinol Metab **85**(7): 2504-8.

Milne, K. L. and H. M. Hayes, Jr. (1981). "Epidemiologic features of canine hypothyroidism." Cornell Vet **71**(1): 3-14.

Mittelman, S. D., G. W. Van Citters, et al. (2000). "Longitudinal compensation for fat-induced insulin resistance includes reduced insulin clearance and enhanced beta-cell response." Diabetes **49**(12): 2116-25.

Mittelman, S. D., G. W. Van Citters, et al. (2002). "Extreme insulin resistance of the central adipose depot in vivo." Diabetes **51**(3): 755-61.

Monzillo, L. U. and O. Hamdy (2003). "Evaluation of insulin sensitivity in clinical practice and in research settings." Nutr Rev **61**(12): 397-412.

Muller, M. J., B. von Schutz, et al. (1986). "Glucoregulatory function of thyroid hormones: interaction with insulin depends on the prevailing glucose concentration." J Clin Endocrinol Metab **63**(1): 62-71.

Nasraway, S. A., Jr. (2006). "Hyperglycemia During Critical Illness." JPEN J Parenter Enteral Nutr **30**(3): 254-258.

Ni, T. C., M. Ader, et al. (1997). "Reassessment of glucose effectiveness and insulin sensitivity from minimal model analysis: a theoretical evaluation of the single-compartment glucose distribution assumption." Diabetes **46**(11): 1813-21.

Okajima, F. and M. Ui (1979). "Metabolism of glucose in hyper- and hypo-thyroid rats in vivo. Glucose-turnover values and futile-cycle activities obtained with 14C- and 3H-labelled glucose." Biochem J **182**(2): 565-75.

Olson, A. L. and J. E. Pessin (1996). "Structure, function, and regulation of the mammalian facilitative glucose transporter gene family." Annu Rev Nutr **16**: 235-56.

Owecki, M., E. Nikisch, et al. (2006). "Hypothyroidism has no impact on insulin sensitivity assessed with HOMA-IR in totally thyroidectomized patients." Acta Clin Belg **61**(2): 69-73.

Pacini, G. and R. N. Bergman (1986). "MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test." Comput Methods Programs Biomed **23**(2): 113-22.

Pacini, G., G. Tonolo, et al. (1998). "Insulin sensitivity and glucose effectiveness: minimal model analysis of regular and insulin-modified FSIGT." Am J Physiol **274**(4 Pt 1): E592-9.

Panciera, D. L. (1994 a). "Hypothyroidism in dogs: 66 cases (1987-1992)." J Am Vet Med Assoc **204**(5): 761-7.

Panciera, D. L. (1994 b). "An echocardiographic and electrocardiographic study of cardiovascular function in hypothyroid dogs." J Am Vet Med Assoc **205**(7): 996-1000.

Panciera, D. L., B. J. Purswell, et al. (2007). "Effect of short-term hypothyroidism on reproduction in the bitch." Theriogenology **68**(3): 316-21.

Pedersen, O., B. Richelsen, et al. (1988). "Characterization of the insulin resistance of glucose utilization in adipocytes from patients with hyper- and hypothyroidism." Acta Endocrinol (Copenh) **119**(2): 228-34.

Perseghin, G., T. B. Price, et al. (1996). "Increased glucose transport-phosphorylation and muscle glycogen synthesis after exercise training in insulin-resistant subjects." N Engl J Med **335**(18): 1357-62.

Peterson, M. E. (1995). "Diagnosis and management of insulin resistance in dogs and cats with diabetes mellitus." Vet Clin North Am Small Anim Pract **25**(3): 691-713.

Prager, R., P. Wallace, et al. (1986). "In vivo kinetics of insulin action on peripheral glucose disposal and hepatic glucose output in normal and obese subjects." J Clin Invest **78**(2): 472-81.

Quon, M. J., C. Cochran, et al. (1994). "Non-insulin-mediated glucose disappearance in subjects with IDDM. Discordance between experimental results and minimal model analysis." Diabetes **43**(7): 890-6.

Randin, J. P., L. Tappy, et al. (1986). "Insulin sensitivity and exogenous insulin clearance in Graves' disease. Measurement by the glucose clamp technique and continuous indirect calorimetry." Diabetes **35**(2): 178-81.

Randle, P. J., P. B. Garland, et al. (1963). "The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus." Lancet **1**: 785-9.

Renauld, A., L. L. Andrade, et al. (1974). "Serum insulin response to glucose infusion in hyperthyroid dogs." Horm Metab Res **6**(5): 400-3.

Renauld, A., J. E. Pinto, et al. (1973). "Studies on the effect of thyroidectomy on the insulin response to hyperglycaemia in the dog." Acta Physiol Lat Am **23**(1): 29-36.

Renauld, A., R. C. Sverdlik, et al. (1974). "Effects of hypothyroidism on serum immunoreactive insulin, free fatty acids and blood sugar in the dog as tested for oral glucose tolerance. Corrective effects of thyroxine therapy." Horm Metab Res **6**(2): 137-41.

Renauld, A., R. C. Sverdlik, et al. (1972). "Studies on the effect of thyroxine replacement therapy on the increased insulin response to hyperglycemia in the thyroidectomized dog." Horm Metab Res **4**(5): 373-6.

Renauld, A., R. C. Sverdlik, et al. (1982). "Blood sugar, serum insulin and serum free fatty acid responses to graded glucose pulses in hypothyroid dogs." Acta Diabetol Lat **19**(2): 97-105.

Rijnberk, A., H. S. Kooistra, et al. (2003). "Endocrine diseases in dogs and cats: similarities and differences with endocrine diseases in humans." Growth Hormone & IGF Research **13**(Supplement 1): S158-S164.

Rochon, C., I. Tauveron, et al. (2003). "Response of glucose disposal to hyperinsulinaemia in human hypothyroidism and hyperthyroidism." Clin Sci (Lond) **104**(1): 7-15.

Rogers, S., M. L. Macheda, et al. (2002). "Identification of a novel glucose transporter-like protein---GLUT-12." Am J Physiol Endocrinol Metab **282**(3): E733-738.

Romero, R., B. Casanova, et al. (2000). "Stimulation of glucose transport by thyroid hormone in 3T3-L1 adipocytes: increased abundance of GLUT1 and GLUT4 glucose transporter proteins." J Endocrinol **164**(2): 187-95.

Rosenfeld, R. G., D. M. Wilson, et al. (1982). "Both human pituitary growth hormone and recombinant DNA-derived human growth hormone cause insulin resistance at a postreceptor site." J Clin Endocrinol Metab **54**(5): 1033-8.

Rossetti, L., A. Giaccari, et al. (1990). "Glucose toxicity." Diabetes Care **13**(6): 610-630.

Saad, M. F., R. L. Anderson, et al. (1994). "A comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. Insulin Resistance Atherosclerosis Study." Diabetes **43**(9): 1114-21.

Scherrer, U., D. Randin, et al. (1994). "Body fat and sympathetic nerve activity in healthy subjects." Circulation **89**(6): 2634-40.

Selman, P. J., J. A. Mol, et al. (1994). "Progestin treatment in the dog. I. Effects on growth hormone, insulin-like growth factor I and glucose homeostasis." Eur J Endocrinol **131**(4): 413-21.

Shah, J. H., G. S. Motto, et al. (1975). "Insulin metabolism in hypothyroidism." Diabetes **24**(10): 922-5.

Shepherd, P. R. and B. B. Kahn (1999). "Glucose transporters and insulin action--implications for insulin resistance and diabetes mellitus." N Engl J Med **341**(4): 248-57.

Stanicka, S., K. Vondra, et al. (2005). "Insulin sensitivity and counter-regulatory hormones in hypothyroidism and during thyroid hormone replacement therapy." Clin Chem Lab Med **43**(7): 715-20.

Tobin, B. L. and D. T. Finegood (1993). "Reduced insulin secretion by repeated low doses of STZ impairs glucose effectiveness but does not induce insulin resistance in dogs." Diabetes **42**(3): 474-83.

Toffolo, G., R. N. Bergman, et al. (1980). "Quantitative estimation of beta cell sensitivity to glucose in the intact organism: a minimal model of insulin kinetics in the dog." Diabetes **29**(12): 979-90.

Torrance, C. J., J. E. deVente, et al. (1997). "Effects of Thyroid Hormone on GLUT4 Glucose Transporter Gene Expression and NIDDM in Rats." Endocrinology **138**(3): 1204-1214.

Torre, D. M., A. M. deLaforcade, et al. (2007). "Incidence and Clinical Relevance of Hyperglycemia in critically Ill Dogs." J Vet Intern Med **21**(5): 971-975.

Trout, K. K., C. Homko, et al. (2007). "Methods of measuring insulin sensitivity." Biol Res Nurs **8**(4): 305-18.

Tuzcu, A., M. Bahceci, et al. (2005). "Subclinical hypothyroidism may be associated with elevated high-sensitive c-reactive protein (low grade inflammation) and fasting hyperinsulinemia." Endocr J **52**(1): 89-94.

Wallace, T. M., J. C. Levy, et al. (2004). "Use and Abuse of HOMA Modeling." Diabetes Care **27**(6): 1487-1495.

Ward, G. M., K. M. Weber, et al. (1991). "A modified minimal model analysis of insulin sensitivity and glucose-mediated glucose disposal in insulin-dependent diabetes." Metabolism **40**(1): 4-9.

Watson, R. T. and J. E. Pessin (2001). "Intracellular Organization of Insulin Signaling and GLUT4 Translocation." Recent Prog Horm Res **56**(1): 175-194.

Weinstein, S. P., J. Watts, et al. (1991). "Thyroid hormone increases muscle/fat glucose transporter gene expression in rat skeletal muscle." Endocrinology **129**(1): 455-64.

Welch, S., S. S. Gebhart, et al. (1990). "Minimal model analysis of intravenous glucose tolerance test-derived insulin sensitivity in diabetic subjects." J Clin Endocrinol Metab **71**(6): 1508-18.

Weyer, C., C. Bogardus, et al. (1999). "The natural history of insulin secretory dysfunction and insulin resistance in the pathogenesis of type 2 diabetes mellitus." Journal of Clinical Investigation **104**(6): 787-794.

Wolfsheimer, K. J. and M. E. Peterson (1991). "Erythrocyte insulin receptors in dogs with spontaneous hyperadrenocorticism." Am J Vet Res **52**(6): 917-21.

Yang, Y. J., J. H. Youn, et al. (1987). "Modified protocols improve insulin sensitivity estimation using the minimal model." Am J Physiol **253**(6 Pt 1): E595-602.

Appendix I: Additional tests

Material and Methods

Growth hormone stimulation and suppression tests

Growth hormone (GH) stimulation and suppression tests were performed 549-775 days after hypothyroidism was induced. Each experiment was performed in the morning, one week apart. Twelve dogs were used (6 /group). On the morning of the experiment, a 20 G indwelling catheter (Becton Dickinson, Franklin Lakes, NJ) was placed in the cephalic vein and animals rested for 30 minutes. All samples were drawn by jugular venipuncture and placed in serum collection tubes (Becton Dickinson, Franklin Lakes, NJ). Two baseline serum samples (4 ml) were drawn, each 15 minutes apart. At time 0, growth hormone releasing hormone (GHRH, 1 µg/kg; Bachem Bioscience Inc., King of Prussia, PA) or Somatostatin (10 µg/kg; Bachem Bioscience Inc., King of Prussia, PA), respectively, was injected through the cephalic catheter and additional samples (3 ml each) were collected at 15, 30, 45, 60 and 90 minutes. Prior to use, somatostatin and GHRH were diluted in sterile water to a final concentration of 20 µg/ml (GHRH) and 100 µg/ml (Somatostatin) and unused aliquots were frozen immediately at -70 °C for future use. All serum samples were allowed to clot for 20 minutes and centrifuged at 1500 x g for 15 min. Serum was separated and frozen in aliquots at –70°C for future assays of GH. In addition, two serum baseline samples, one from each experiment, were also used for determination of Insulin-like growth factor I (IGF-I). Two additional sets of two baseline

samples, taken 15 minutes apart, were obtained on separate days, so that there was a total of 8 baseline samples.

Analysis of Growth hormone and IGF-I

Serum growth hormone concentrations were determined by a commercially available radioimmunoassay for canine/porcine GH (PGH-46HK, Linco Research, St.Charles, MS). The assay has been validated for canine serum. The average of the 8 baseline GH concentrations per dog was used for statistical analysis (basal GH).
Serum (2 samples per animal) was submitted to a diagnostic laboratory to have insulin-like growth factor I (IGF-I) determined by a commercially available radioimmunoassay. The average IGF-I concentrations were used for statistical analysis.

Urine cortisol and catecholamine concentrations

Two morning urine samples were collected by cystocentesis in 7 euthyroid and 8 hypothyroid dogs for urine cortisol to creatinine ratio determinations. The analysis was performed in an outside diagnostic laboratory. For catecholamine to creatinine ratios, two morning samples from 5 hypothyroid and 8 samples from euthyroid dogs were obtained. Each sample was collected on separate days, at least 7 days apart from any other experiment. Urine for cortisol and creatinine measurements was frozen at –70°C for future assays. For catecholamine:creatinine ratios, one aliquot was frozen immediately, while another aliquot (5-8 ml) from the same sample was acidified with concentrated hydrochloric acid. Acidified samples were frozen at -20 °C and analyzed at the same time to avoid interassay variation. Urinary free epinephrine, norepinephrine, metanephrine, and normetanephrine concentrations were determined by high-pressure liquid chromatography (HPLC) and expressed as ratios to urine creatinine concentrations (in

nmol/mmol), which were measured on the corresponding non-acidified samples. The average of the two samples per animal was used for statistical comparison.

DEXA Scans

Eight hypothyroid dogs and 8 euthyroid dogs underwent a dual energy x-ray absorptiometry (DEXA) scan procedure to determine total body mass in kg (i.e. body weight), lean body mass (kg), absolute (kg) and percent body fat as well as bone mineral density. Animals were anesthetized with isoflurane (Abbott Laboratories, Abbott Park, IL) only, as part of another experiment conducted on the same day. They were placed in sternal recumbency and the procedure performed with a commercially available DEXA scanner (Lunar Prodigy Advance, GE Healthcare, Madison, WI), using a standard scan mode of 1.8 Gy. In addition to the aforementioned parameters, a region of interest was drawn around the abdomen and percent of abdominal fat calculated.

Statistical analysis

Experimental data are reported and analyzed as mentioned above, and P-values < 0.05 were considered to be significant. Some data had to be log-transformed to achieve normality. Data from the GH stimulation and suppression tests was used to calculate areas-under-the curve (AUC) by applying the trapezoidal rule. In addition, peak and nadir GH concentrations as well as difference from peak and nadir to baseline were calculated. Growth hormone baseline was defined as the average of the two baseline samples obtained in each test. The means of each measurement were used to test for differences between groups during stimulation and suppression tests.

By using correlation and simple regression analyses, data of each group was analyzed for relationships between basal insulin and S_I as well as between variables derived from the

minimal model analysis (S_I, S_G, AIR_g, DI, basal insulin (IB) and basal glucose (GB) and parameters from the GH analysis, cortisol to creatinine and catecholamine to creatinine ratios (log-transformed), IGF-I concentrations and the DEXA scan procedure. The Pearson's correlation coefficient was calculated for normally distributed data, whereas the Spearman's rank correlation coefficient was calculated for nonparametric data. In addition to the mean, 95% confidence intervals of the correlation coefficients are reported (Table 1).

Results

No important association was found between any of the parameters analyzed (Table 1).

Results of the t-tests are presented in Table 2 as means (MN) and standard deviation (SD). *P*-values are listed for each variable.

Raw data of each test for each animal (see material and methods in Chapter II and Appendix I) is presented in Tables 3 and 4.

List of Figures and Tables

Figures

Figure 1: Average insulin concentrations (+/ SD) during FSIGT in 8 hypothyroid and 7 control dogs

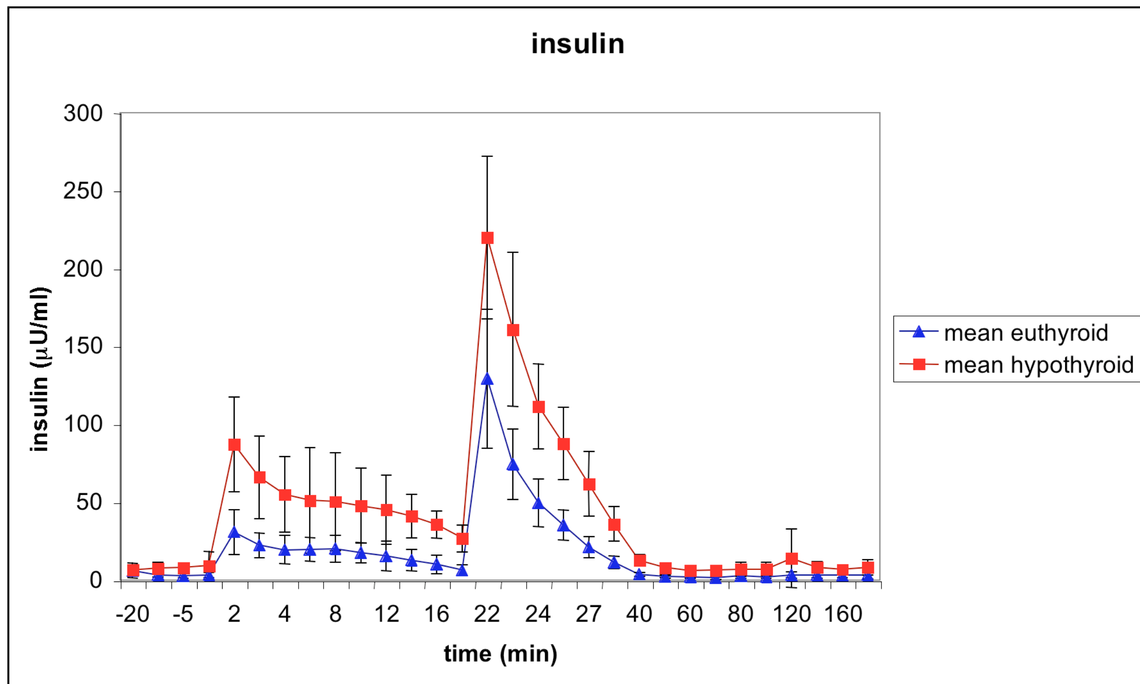
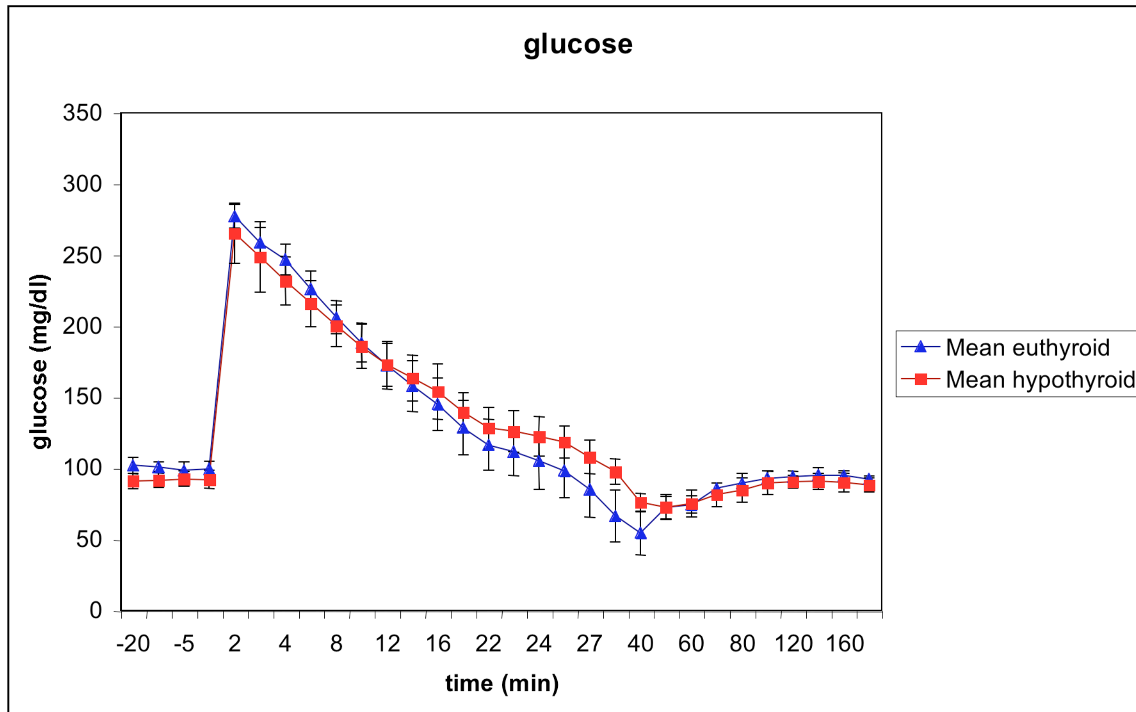


Figure 2: Average glucose concentrations (+/- SD) during FSIGT of 8 hypothyroid and 7 euthyroid dogs



Tables

Table 1: Correlation coefficients, 95% confidence intervals and P-values for each pair of variables analyzed as described material and methods. Data divided by groups.

Control group (n = 7)

					Confidence Limits		
Variable	With variable	N	Sample Correlation	Fisher's z	Lower	Upper	p Value
AIRg	CortisolMN	6	0.018	0.018	-0.805	0.818	0.975
DI	CortisolMN	6	-0.152	-0.153	-0.858	0.752	0.791
SI	CortisolMN	6	-0.354	-0.370	-0.905	0.642	0.522
Sg	CortisolMN	6	-0.132	-0.133	-0.852	0.761	0.818
GB	CortisolMN	6	0.671	0.813	-0.308	0.960	0.159
IB	CortisolMN	6	-0.177	-0.179	-0.864	0.741	0.757
AIRg	IGF1MN	7	0.662	0.796	-0.182	0.944	0.112
DI	IGF1MN	7	0.423	0.452	-0.484	0.892	0.366
SI	IGF1MN	7	-0.080	-0.080	-0.786	0.716	0.872
Sg	IGF1MN	7	-0.332	-0.345	-0.868	0.561	0.490
GB	IGF1MN	7	0.272	0.279	-0.605	0.851	0.576
IB	IGF1MN	7	0.404	0.429	-0.501	0.887	0.391
AIRg	weight	7	-0.604	-0.700	-0.933	0.273	0.161
DI	weight	7	-0.704	-0.875	-0.952	0.105	0.080
SI	weight	7	-0.332	-0.345	-0.868	0.562	0.491
Sg	weight	7	0.786	1.061	0.080	0.967	0.034
GB	weight	7	-0.008	-0.008	-0.757	0.749	0.987
IB	weight	7	-0.493	-0.541	-0.909	0.413	0.280
AIRg	PctTissue	7	-0.719	-0.906	-0.955	0.074	0.070
DI	PctTissue	7	-0.753	-0.980	-0.961	0.000	0.050
SI	PctTissue	7	-0.259	-0.265	-0.847	0.614	0.596
Sg	PctTissue	7	0.765	1.008	0.028	0.963	0.044
GB	PctTissue	7	-0.160	-0.161	-0.815	0.674	0.747
IB	PctTissue	7	-0.612	-0.713	-0.934	0.261	0.154
AIRg	tissueKg	7	-0.598	-0.690	-0.932	0.282	0.167
DI	tissueKg	7	-0.702	-0.871	-0.952	0.108	0.081
SI	tissueKg	7	-0.334	-0.347	-0.869	0.560	0.487
Sg	tissueKg	7	0.789	1.070	0.090	0.967	0.032
GB	tissueKg	7	-0.018	-0.018	-0.761	0.745	0.971
IB	tissueKg	7	-0.489	-0.534	-0.908	0.418	0.285
AIRg	fatKg	7	-0.715	-0.898	-0.954	0.082	0.073
DI	fatKg	7	-0.750	-0.972	-0.961	0.008	0.052

SI	fatKg	7	-0.255	-0.261	-0.846	0.617	0.602
Sg	fatKg	7	0.818	1.152	0.170	0.972	0.021
GB	fatKg	7	-0.158	-0.160	-0.814	0.675	0.750
IB	fatKg	7	-0.590	-0.678	-0.930	0.293	0.175
AIRg	leanKg	7	0.455	0.491	-0.453	0.900	0.326
DI	leanKg	7	0.356	0.372	-0.543	0.874	0.457
SI	leanKg	7	-0.034	-0.034	-0.767	0.738	0.946
Sg	leanKg	7	-0.350	-0.366	-0.873	0.547	0.465
GB	leanKg	7	0.286	0.295	-0.595	0.855	0.556
IB	leanKg	7	0.383	0.404	-0.520	0.882	0.419
AIRg	BMCKg	7	-0.598	-0.689	-0.931	0.283	0.168
DI	BMCKg	7	-0.560	-0.633	-0.924	0.334	0.206
SI	BMCKg	7	-0.178	-0.180	-0.821	0.664	0.719
Sg	BMCKg	7	0.563	0.638	-0.330	0.924	0.202
GB	BMCKg	7	0.247	0.252	-0.622	0.843	0.615
IB	BMCKg	7	-0.510	-0.563	-0.913	0.394	0.260
AIRg	fatFreeKg	5	0.082	0.082	-0.863	0.899	0.907
DI	fatFreeKg	5	0.052	0.052	-0.870	0.893	0.942
SI	fatFreeKg	5	0.024	0.024	-0.877	0.887	0.973
Sg	fatFreeKg	5	0.482	0.526	-0.696	0.957	0.457
GB	fatFreeKg	5	-0.304	-0.314	-0.935	0.790	0.657
IB	fatFreeKg	5	0.203	0.206	-0.828	0.920	0.771
AIRg	AbdFatPctMN	7	-0.607	-0.704	-0.933	0.269	0.159
DI	AbdFatPctMN	7	-0.643	-0.763	-0.941	0.214	0.127
SI	AbdFatPctMN	7	-0.536	-0.598	-0.918	0.364	0.232
Sg	AbdFatPctMN	7	0.500	0.549	-0.406	0.910	0.272
GB	AbdFatPctMN	7	0.270	0.277	-0.606	0.850	0.579
IB	AbdFatPctMN	7	-0.857	-1.282	-0.979	-0.294	0.010
AIRg	NorepiToCreaMN	7	0.143	0.144	-0.684	0.809	0.774
DI	NorepiToCreaMN	7	0.357	0.374	-0.542	0.875	0.455
SI	NorepiToCreaMN	7	0.500	0.549	-0.406	0.910	0.272
Sg	NorepiToCreaMN	7	0.036	0.036	-0.737	0.768	0.943
GB	NorepiToCreaMN	7	0.054	0.054	-0.729	0.776	0.914
IB	NorepiToCreaMN	7	-0.214	-0.218	-0.833	0.642	0.663
AIRg	EpiToCreaMN	7	-0.071	-0.072	-0.782	0.720	0.886
DI	EpiToCreaMN	7	0.107	0.108	-0.703	0.796	0.830
SI	EpiToCreaMN	7	0.429	0.458	-0.479	0.893	0.360
Sg	EpiToCreaMN	7	0.500	0.549	-0.406	0.910	0.272
GB	EpiToCreaMN	7	-0.054	-0.054	-0.776	0.729	0.914
IB	EpiToCreaMN	7	-0.536	-0.598	-0.918	0.364	0.232
AIRg	NormetaToCreaMN	7	-0.071	-0.072	-0.782	0.720	0.886
DI	NormetaToCreaMN	7	0.250	0.255	-0.620	0.844	0.609
SI	NormetaToCreaMN	7	0.464	0.503	-0.444	0.902	0.315
Sg	NormetaToCreaMN	7	-0.143	-0.144	-0.809	0.684	0.774
GB	NormetaToCreaMN	7	0.054	0.054	-0.729	0.776	0.914
IB	NormetaToCreaMN	7	-0.179	-0.181	-0.821	0.664	0.718
AIRg	MetaToCreaMN	7	-0.250	-0.255	-0.844	0.620	0.609

DI	MetaToCreaMN	7	0.036	0.036	-0.737	0.768	0.943
SI	MetaToCreaMN	7	0.321	0.333	-0.569	0.865	0.505
Sg	MetaToCreaMN	7	0.286	0.294	-0.595	0.855	0.557
GB	MetaToCreaMN	7	0.036	0.036	-0.737	0.768	0.943
IB	MetaToCreaMN	7	-0.571	-0.650	-0.926	0.319	0.194
AIRg	GhBaseline	7	-0.071	-0.072	-0.782	0.720	0.886
DI	GhBaseline	7	-0.071	-0.072	-0.782	0.720	0.886
SI	GhBaseline	7	0.143	0.144	-0.684	0.809	0.774
Sg	GhBaseline	7	-0.250	-0.255	-0.844	0.620	0.609
GB	GhBaseline	7	0.595	0.685	-0.287	0.931	0.171
IB	GhBaseline	7	-0.071	-0.072	-0.782	0.720	0.886
SI	IB	7	0.179	0.181	-0.664	0.821	0.718

Hypothyroid group: (n = 8):

					Confidence Limits		
Variable	With variable	N	Sample Correlation	Fisher's z	Lower	Upper	p Value
AIRg	CortisolMN	8	0.385	0.406	-0.438	0.857	0.364
DI	CortisolMN	8	0.527	0.586	-0.283	0.898	0.190
SI	CortisolMN	8	0.505	0.556	-0.310	0.892	0.214
Sg	CortisolMN	8	0.614	0.715	-0.160	0.920	0.110
GB	CortisolMN	8	0.324	0.336	-0.493	0.837	0.452
IB	CortisolMN	8	0.003	0.003	-0.703	0.706	0.994
AIRg	IGF1MN	8	-0.571	-0.650	-0.910	0.223	0.146
DI	IGF1MN	8	-0.568	-0.644	-0.909	0.228	0.150
SI	IGF1MN	8	0.033	0.033	-0.688	0.721	0.942
Sg	IGF1MN	8	-0.509	-0.561	-0.893	0.305	0.209
GB	IGF1MN	8	0.554	0.624	-0.247	0.905	0.163
IB	IGF1MN	8	0.046	0.046	-0.681	0.727	0.917
AIRg	weight	8	-0.157	-0.158	-0.776	0.616	0.723
DI	weight	8	-0.288	-0.296	-0.825	0.523	0.508
SI	weight	8	-0.545	-0.611	-0.903	0.259	0.172
Sg	weight	8	-0.085	-0.085	-0.745	0.659	0.849
GB	weight	8	-0.057	-0.057	-0.732	0.675	0.899
IB	weight	8	0.293	0.302	-0.519	0.827	0.499
AIRg	PctTissue	8	0.173	0.174	-0.606	0.782	0.696
DI	PctTissue	8	0.053	0.053	-0.677	0.731	0.905
SI	PctTissue	8	-0.379	-0.399	-0.855	0.444	0.372
Sg	PctTissue	8	0.426	0.455	-0.398	0.870	0.309
GB	PctTissue	8	-0.285	-0.293	-0.824	0.525	0.512
IB	PctTissue	8	0.445	0.479	-0.378	0.875	0.284
AIRg	tissueKg	8	-0.157	-0.158	-0.776	0.616	0.724
DI	tissueKg	8	-0.297	-0.306	-0.828	0.516	0.494

SI	tissueKg	8	-0.572	-0.650	-0.910	0.223	0.146
Sg	tissueKg	8	-0.098	-0.098	-0.751	0.652	0.826
GB	tissueKg	8	-0.069	-0.069	-0.738	0.668	0.877
IB	tissueKg	8	0.306	0.317	-0.508	0.832	0.479
AIRg	fatKg	8	0.127	0.127	-0.635	0.763	0.776
DI	fatKg	8	-0.012	-0.012	-0.711	0.698	0.978
SI	fatKg	8	-0.473	-0.514	-0.883	0.348	0.251
Sg	fatKg	8	0.369	0.387	-0.454	0.852	0.387
GB	fatKg	8	-0.276	-0.284	-0.821	0.532	0.526
IB	fatKg	8	0.478	0.521	-0.341	0.885	0.244
AIRg	leanKg	8	-0.229	-0.233	-0.804	0.567	0.602
DI	leanKg	8	-0.158	-0.159	-0.776	0.615	0.721
SI	leanKg	8	0.183	0.185	-0.599	0.786	0.678
Sg	leanKg	8	-0.456	-0.492	-0.878	0.366	0.271
GB	leanKg	8	0.262	0.268	-0.543	0.816	0.548
IB	leanKg	8	-0.338	-0.352	-0.842	0.481	0.431
AIRg	BMCKg	8	-0.070	-0.070	-0.738	0.668	0.875
DI	BMCKg	8	-0.002	-0.002	-0.706	0.704	0.996
SI	BMCKg	8	0.134	0.135	-0.630	0.766	0.763
Sg	BMCKg	8	0.235	0.239	-0.563	0.806	0.593
GB	BMCKg	8	0.148	0.149	-0.621	0.772	0.738
IB	BMCKg	8	-0.101	-0.102	-0.752	0.650	0.820
AIRg	fatFreeKg	7	0.280	0.288	-0.599	0.853	0.565
DI	fatFreeKg	7	-0.118	-0.119	-0.800	0.697	0.812
SI	fatFreeKg	7	-0.640	-0.759	-0.940	0.218	0.129
Sg	fatFreeKg	7	0.167	0.168	-0.670	0.817	0.736
GB	fatFreeKg	7	-0.388	-0.410	-0.883	0.516	0.413
IB	fatFreeKg	7	0.161	0.162	-0.674	0.815	0.745
AIRg	AbdFatPctMN	8	-0.095	-0.096	-0.750	0.653	0.831
DI	AbdFatPctMN	8	0.429	0.458	-0.396	0.870	0.306
SI	AbdFatPctMN	8	0.714	0.896	0.019	0.944	0.045
Sg	AbdFatPctMN	8	-0.286	-0.294	-0.824	0.525	0.511
GB	AbdFatPctMN	8	0.452	0.488	-0.370	0.877	0.275
IB	AbdFatPctMN	8	-0.214	-0.218	-0.798	0.578	0.626
AIRg	NorepiToCreaMN	4	0.400	0.424	-0.911	0.983	0.672
DI	NorepiToCreaMN	4	0.400	0.424	-0.911	0.983	0.672
SI	NorepiToCreaMN	4	0.200	0.203	-0.942	0.974	0.839
Sg	NorepiToCreaMN	4	0.800	1.099	-0.697	0.996	0.272
GB	NorepiToCreaMN	4	-0.200	-0.203	-0.974	0.942	0.839
IB	NorepiToCreaMN	4	-0.400	-0.424	-0.983	0.911	0.672
AIRg	EpiToCreaMN	4	-0.200	-0.203	-0.974	0.942	0.839
DI	EpiToCreaMN	4	0.200	0.203	-0.942	0.974	0.839
SI	EpiToCreaMN	4	0.400	0.424	-0.911	0.983	0.672
Sg	EpiToCreaMN	4	0.600	0.693	-0.853	0.990	0.488
GB	EpiToCreaMN	4	0.400	0.424	-0.911	0.983	0.672
IB	EpiToCreaMN	4	-0.200	-0.203	-0.974	0.942	0.839
AIRg	NormetaToCreaMN	4	0.400	0.424	-0.911	0.983	0.672

DI	NormetaToCreaMN	4	-0.400	-0.424	-0.983	0.911	0.672
SI	NormetaToCreaMN	4	-0.800	-1.099	-0.996	0.697	0.272
Sg	NormetaToCreaMN	4	0.800	1.099	-0.697	0.996	0.272
GB	NormetaToCreaMN	4	-0.800	-1.099	-0.996	0.697	0.272
IB	NormetaToCreaMN	4	0.400	0.424	-0.911	0.983	0.672
AIRg	MetaToCreaMN	4	0.400	0.424	-0.911	0.983	0.672
DI	MetaToCreaMN	4	-0.400	-0.424	-0.983	0.911	0.672
SI	MetaToCreaMN	4	-0.800	-1.099	-0.996	0.697	0.272
Sg	MetaToCreaMN	4	0.800	1.099	-0.697	0.996	0.272
GB	MetaToCreaMN	4	-0.800	-1.099	-0.996	0.697	0.272
IB	MetaToCreaMN	4	0.400	0.424	-0.911	0.983	0.672
AIRg	GhBaseline	8	-0.167	-0.168	-0.780	0.610	0.707
DI	GhBaseline	8	-0.024	-0.024	-0.716	0.692	0.958
SI	GhBaseline	8	-0.071	-0.072	-0.739	0.667	0.873
Sg	GhBaseline	8	-0.857	-1.282	-0.974	-0.385	0.004
GB	GhBaseline	8	-0.238	-0.243	-0.807	0.561	0.587
IB	GhBaseline	8	-0.238	-0.243	-0.807	0.561	0.587
SI	IB	8	-0.595	-0.686	-0.916	0.189	0.125

Table 2: Mean (MN) and standard deviation (SD) per group of additional tests described in Appendix I.

	MN Control	SD Control	MN Hypothyroid	SD Hypothyroid	P-value
Cortisol:Creatinine (mmol/mmol)	0.01	0.007	0.01	0.007	0.591
GH basal (ng/ml)	2.24	1.346	3.98	1.247	0.022
GH stimulation test:					
GH peak	3.05	2.519	5.80	5.776	0.325
Diff Peak to baseline	1.85	2.220	2.38	4.580	0.806
% Diff Peak to baseline	151.03	169.930	107.61	173.110	0.670
AUC	159.25	107.420	264.81	205.870	0.292
GH suppression test:					
Nadir	0.98	0.861	2.02	1.306	0.137
Diff Nadir to baseline	-0.48	0.410	-1.16	0.798	0.092
AUC	116.56	75.041	214.94	110.920	0.102
IGF-1 (nmol/l)	11.14	4.625	17.56	5.395	0.029
BW (kg)	10.35	1.226	11.93	0.586	0.006
Tissue (kg)	10.05	1.197	11.58	0.567	0.006
BMC (kg)	0.31	0.036	0.34	0.031	0.048
% fat	36.11	11.104	42.50	8.980	0.240
Fat (kg)	5.07	1.471	5.82	1.064	0.086
Abd fat %	34.61	8.784	47.46	3.643	0.002
Fat free (kg)	6.77	0.602	6.74	0.677	0.935
Lean (kg)	6.33	0.738	6.65	0.985	0.501
log Norepi:Crea (nmol/mmol)	1.71	0.392	3.02	0.518	0.001
log Epi:Crea (nmol/mmol)	1.50	0.490	2.30	0.593	0.038
log Normeta:Crea (nmol/mmol)	3.28	0.408	4.62	0.293	0.000
log Meta:Crea (nmol/mmol)	3.03	0.406	3.66	0.502	0.047

Table 3: Data from all analyses as described in material and methods (Chapter II and Appendix I), divided by animal and group.

Control group:

Animal ID	MAV	MCN *	MAS	MBL	MAW	MBH	MAY	DMBV
AIRg	184	176	113	150	96	236	222	114
DI	6070	23800	2590	3650	1780	5810	3680	2850
SI	33.10	135.00	22.90	24.40	18.50	24.60	16.60	24.90
Sg	0.042	0.105	0.053	0.044	0.036	0.028	0.042	0.052
GB	91.80	97.50	98.00	98.00	99.50	99.30	95.30	92.60
IB	3.70	2.11	2.24	2.46	3.09	8.74	5.85	3.50
Cortisol:Crea MN	0.007	0.014	0.014	0.024	0.013	0.012		0.005
IGF-1 MN	9.00	7.50	8.50	18.00	6.00	15.00	14.50	7.00
Weight (kg)	9.07	10.41	12.43	9.81	10.12	9.15	10.40	11.48
% Fat	23.9	6.10	41.10	41.3	36.60	21.90	33.7	54.30
fat (kg)	2.10	0.62	4.95	3.93	3.58	1.94	3.41	6.07
lean (kg)	6.69	9.43	7.10	5.59	6.21	6.93	6.71	5.11
BMC (kg)	0.29	0.37	0.38	0.29	0.31	0.28	0.29	0.30
fat free (kg)	6.97	9.80	7.48	5.88	6.53		7.00	
Abd fat %	25.40	6.90	43.70	45.90	39.25	24.95	35.70	27.40
Norepi/Crea MN	8.06	7.10	5.98	8.17	4.96	6.39	2.60	4.78
Epi/Crea MN	5.10	8.65	7.00	9.96	3.05	4.16	2.55	3.18
Normeta/Crea MN	43.09	32.71	25.87	37.81	31.33	26.33	12.61	20.88
Meta/Crea MN	34.00	20.01	20.04	37.32	19.79	17.40	12.86	14.34
GH Baseline	2.08	1.90	3.04	1.70	2.13	4.75	0.50	1.49

* indicates animal whose values obtained from minimal model analysis were excluded from statistical analyses (both t-tests and correlation procedures).

Hypothyroid group:

Animal ID	MCJ	MCZ	MBS	MBW	MAP	MCC	BMBV	MBN	MCI
AIRg	379	273	989	228	485	376	673	266	
DI	2590	1060	5310	1330	2570	1310	2370	1410	
SI	6.83	3.90	5.37	5.84	5.30	3.48	3.52	5.27	
Sg	0.031	0.039	0.053	0.053	0.041	0.035	0.058	0.032	
GB	94.30	91.10	85.40	97.10	90.10	91.60	85.50	85.30	
IB	4.01	10.80	5.98	12.10	5.35	12.20	8.72	6.13	
Cortisol:Crea MN	0.016	0.006	0.024	0.026	0.012	0.010	0.013	0.007	
IGF-1 MN	25.5	18	7	16.5	19	21.5	18.5	14.5	
Weight (kg)	11.27	13.08	11.67	11.52	12.48	11.98	11.78	11.62	
% Fat	22.00	40.50	49.70	49.10	45.10	46.7	40.40	46.50	
fat (kg)	2.41	5.14	5.63	5.48	5.45	5.46	4.62	5.25	
lean (kg)	8.53	7.56	5.70	5.68	6.64	6.22	6.82	6.04	
BMC (kg)	0.33	0.38	0.34	0.36	0.39	0.30	0.34	0.33	
fat free (kg)	6.13	7.94		6.03	7.03	6.52	7.16	6.37	
Abd fat %	51.10	41.70	50.60	50.75	46.55	48.80	42.65	47.50	
Norepi/Crea MN	15.95			25.85		11.48	37.11		20.61
Epi/Crea MN	6.62			18.07		5.51	15.28		32.86
Normeta/Crea MN	71.20			90.64		117.85	138.43		63.54
Meta/Crea MN	22.85			28.26		57.08	62.37		45.72
GH Baseline	4.65	4.11	4.01	1.98	3.90	4.16	2.83	6.23	

Table 4: Growth hormone (GH) concentrations (in ng/ml) of control and hypothyroid dogs during stimulation and suppression tests as well as peak and nadir GH concentrations, differences (absolute and percent) of peak and nadir to average (AVE) basal GH concentrations, and area under the curve (AUC).

Control group:

	Animal ID	MCN	MAS	MBL	MAW	MAY	DMBV
Stimulation	-15	1.1	2.6	<1	1.4	<1	1.3
	0	1	2.3	<1	1.4	<1	1.3
	AVE basal	1.05	2.45	0.5	1.4	0.5	1.3
	15	3	4.1	<1	1.9	<1	7.4
	30	1.7	4.3	1.2	1.2	<1	3.3
	45	1.4	2.9	<1	1.6	<1	2.3
	60	1.6	2.9	<1	1.4	<1	1.7
	90	<1.0	3.3	<1	1.7	<1	1.9
	Peak	3	4.3	1.2	1.9	0.5	7.4
	Diff Peak-base	1.95	1.85	0.7	0.5	0	6.1
	% Diff Peak-base	185.71	75.51	140.00	35.71	0.00	469.23
	AUC	142.88	302.63	55.50	138.00	45.00	271.50
Suppression	-15	1.1	2.7	1	1.6	<1	1.8
	0	1.1	2.8	<1.0	1.2	<1	2.2
	AVE baselines	1.1	2.75	1	1.4	0.5	2
	15	1.1	2.4	<1.0	1.4	<1	1.5
	30	1.2	2.7	<1.0	1	<1	2
	45	1	3.2	<1.0	1.7	<1	2
	60	1.1	2.8	<1.0	1.2	<1	1.1
	90	1	2.5	<1.0	1.4	<1	1.6
	Nadir	1	2.4	0	0.5	0.5	1.5
	Diff low-base	-0.1	-0.35	-1	-0.9	0	-0.5
	%Diff low-peak	-9.09	-12.73	-100.00	-64.29	0.00	-25.00
	AUC	97.50	245.63	45.00	120.00	45.00	146.25

Hypothyroid group:

	Animal ID	MCJ	MCZ	MBS	MBW	MCC	BMBV
Stimulation	-15	2.2	2.6	1.8	<1	3.5	2.9
	0	1.9	2.5	2.3	<1	3.1	2.3
	AVE basal	2.05	2.55	2.05	0.5	3.3	2.6
	15	1.6	4.3	1.6	<1	3.4	14.2
	30	1.8	4.5	1.5	<1	3.2	9.4
	45	1.6	3.8	1.4	<1	3	6.4
	60	1.6	2.9	1.3	<1	3.4	5
	90	1.4	4.1	1.3	1.1	3.3	2.9
	Peak	none	4.5	none	1.1	3.4	14.2
	Diff Peak-base	0	1.95	0	0.6	0.1	11.6
	% Diff Peak-base	0.00	76.47	0.00	120.00	3.03	446.15
	AUC	147.375	334.875	131.625	54	295.5	625.5
Suppression	-15	5.4	3.4	2	<1	3.3	5.3
	0	3.6	3.1	<1.0	1.5	3.2	3.8
	AVE baselines	4.5	3.25	2	1.5	3.25	4.55
	15	3.3	3.8	<1.0	<1	3.8	3.7
	30	2.4	3.5	<1.0	1.3	3	3.7
	45	1.9	3.2	<1.0	1.1	2.7	3.7
	60	2.3	2.8	<1.0	1.9	2.8	3.8
	90	<1	3.2	<1.0	1.1	3.2	3.7
	Nadir	1.9	2.8	0.5	0.5	2.7	3.7
	Diff low-base	-2.6	-0.45	-1.5	-1	-0.55	-0.85
	%Diff low-peak	-57.78	-13.85	-75.00	-66.67	-16.92	-18.68
	AUC	207.00	292.88	56.25	114.00	277.88	341.63