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Dolphins as animal models for type 2 diabetes: Sustained, post-prandial hyperglycemia and hyperinsulinemia

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ABSTRACT

There is currently no known natural animal model that fully complements type 2 diabetes in humans. Criteria for a true natural animal model include the presence of a fasting hyperglycemia, evidence of insulin resistance, and pathologies matching that reported in humans. To investigate the bottlenose dolphin (*Tursiops truncatus*) as a comparative model for type 2 diabetes in humans, hourly plasma and urine chemistry changes, including glucose, were analyzed among five healthy, adult dolphins for 24 h following ingestion of 2.5–3.5 kg of mackerel or 2–3 L of 10% dextrose in ionosol. Fasting and 2 h post-prandial insulin levels were also determined among five adult dolphins to assess the presence of hyperinsulinemia. Finally, a case-control study compared insulin and glucagon levels among dolphins with and without iron overload, a condition associated with insulin resistance in humans. Both protein and dextrose meals caused significant increases in plasma glucose during the 0–5 h post-prandial period; dolphins fed dextrose demonstrated a sustained hyperglycemia lasting 5–10 h. Fasting plasma insulin levels among healthy dolphins mimicked those found in humans with some insulin resistance. Dolphins with hemochromatosis had higher post-prandial plasma insulin levels compared to controls. We conclude that bottlenose dolphins can demonstrate metabolic responses consistent with type 2 diabetes, specifically sustained hyperglycemia and hyperinsulinemia. Understanding more about how and why dolphins have a diabetes-like metabolism may provide new research avenues for diabetes in humans.

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1. Introduction

According to the World Health Organization, approximately 5% of all deaths globally are due to diabetes. Without action, deaths from diabetes are likely to increase by more than 50% over the next 10 years (World Health Organization and Diabetes, 2009). Of people with diabetes, 90–95% have type 2 diabetes, previously called insulin-resistant or adult onset diabetes. A single, natural species has not been identified that fully complements type 2 diabetes in humans (Cefalu, 2006; Kaplan and Wagner, 2006). Discovery of such a model may lead to novel means of preventing, treating, and curing this disease for people.

When comparing common bottlenose dolphins (*Tursiops truncatus*) that are fasted overnight to those recently fed, overnight fasted dolphins demonstrate changes in platelet counts and serum chemistries that mimic those of people with type 2 diabetes (Venn-Watson and Ridgway, 2007). Changes which parallel those found in people with type 2 diabetes include sustained increases in glucose; increased platelets (Sterner et al., 1998); increased serum gamma-glutamyl transpeptidase (Andre et al., 2006) and alkaline phosphatase (Maxwell et al., 1986); decreased serum uric acid (Nan et al., 2007); and shifts toward a metabolic acidodic state (Androgue et al., 1982). The key difference found between dolphins and people with diabetes, however, is that dolphins appear to turn a diabetes-like state on and off with overnight fasting and daily feeding, respectively.

Criteria for diabetes in humans includes random blood glucose levels greater than 200 mg/dl, fasting glucose levels greater than 126 mg/dl, and/or glucose levels greater than 200 mg/dl following an oral glucose tolerance test (Alberti and Zimmet, 1998). Type 2 is typically differentiated from type 1 diabetes from patient history, but insulin resistance and accompanying hyperinsulinemia in type 2 diabetes may be detected. Criteria have been suggested to assess whether or not an animal model fully complements type 2 diabetes in humans (Cefalu, 2006; Kaplan and Wagner, 2006). These criteria include demonstrated sustained post-prandial hyperglycemia, disease complications similar to that seen in people with type 2 diabetes, and a shared evolutionary history of disease. Current animal models that have provided insight into type 2 diabetes include mice, desert rodents, cats, pigs, and old world monkeys (Bellinger et al., 2006; Henson and O'Brien, 2006; Neubauer and Kulkarni, 2006; Shafrir et al., 2006; Wagner et al., 2006). While these animal models mimic parts of type 2 diabetes, none meet all the criteria.

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Physiological similarities identified only in primates and cetaceans (toothed whales, porpoises, and dolphins) exist that support a shared drive for common glucose metabolism. Of all terrestrial and aquatic animals tested, only primates and cetaceans have red blood cells that are 'extraordinarily' permeable to glucose (Craik et al., 1998). Humans and bottlenose dolphins also share high encephalization quotients (EQ) Marino, 1998, a measurement of actual brain size compared to the expected brain size given the body mass (Jerison, 1973). EQs for humans, bottlenose dolphins, and chimpanzees are 7.4, 5.3, and 2.5, respectively. Given that large brains require readily available blood glucose in order to function, it is hypothesized that humans and dolphins have high blood glucose carrying capacity in their blood to support their shared large brain size (Goodwin, 1956).

Dolphins and humans are susceptible to hemochromatosis, also called iron overload, which is responsive to phlebotomy treatment (Johnson et al., 2009). Ferritin is the true measure of iron in blood, and high blood ferritin is reflected in iron overload. In humans, the severity of insulin resistance is associated with increasing serum ferritin levels and hemochromatosis (Wrede et al., 2006). Further, the presence of diabetes mellitus has been identified as a primary risk factor for increased ferritin levels in humans with chronic hepatitis C (D'Souza et al., 2005).

To further assess the bottlenose dolphin as a model for type 2 diabetes in humans, we analyzed hourly urine and plasma glucose changes among healthy dolphins fed either a high protein fish meal or dextrose. We also measured fasting plasma insulin levels among healthy dolphins and compared fasting and 2 h post-prandial insulin levels among dolphins with and without hemochromatosis. Our study results were compared to those found in humans with and without insulin resistance and type 2 diabetes.

2. Methods

2.1. Ethical statement

Dolphins involved in this study were part of the U.S. Navy Mammal Program (MMP) population. The MMP is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and adheres to the national standards of the United States Public Health Service Policy on the Humane Care and Use of Laboratory Animals and the Animal Welfare Act. As required by the Department of Defense, the MMP's animal care and use program is routinely reviewed by an Institutional Animal Care and Use Committee (IACUC) and the Navy Bureau of Medicine. The protein and dextrose feeding studies were conducted in 1970 and followed the rules applicable to animal research at that time; to minimize unnecessary duplication of effort, the authors used raw, archived datasets from these studies to address our current study hypothesis. The study protocol used to determine fasting and post-prandial plasma insulin measurements among the current MMP dolphin population was reviewed and approved by the MMP IACUC and the Navy Bureau of medicine.

2.2. Protein and dextrose feeding studies

Six healthy adult bottlenose dolphins were included in nine feeding trials. Animals were fasted, fed 2–3 L 10% dextrose in ionosol, or fed 4.5–5.4 kg Spanish mackerel (Table 1). Health was determined by behavior, appetite, and clinical blood values (Ridgway et al., 1970). All animals were fasted 12 h overnight before the start of each study. The animals were taken from their seawater habitat in a fleece-lined transport sling. They were placed on their sides on a soft rubber pad. An initial blood sample was taken from the central vessels of the fluke. A standard 8–14 Fr. clinical urinary catheter with inflatable cuff was inserted through the urethra into the urinary bladder. The cuff was inflated with 15–20 ml of normal saline to retain the catheter in the bladder throughout the study. An initial urine sample was taken and the collection end of the catheter was pulled through a small central hole in the transport sling and connected to a length of tubing. The dolphin was then lifted into a transport container that had been partially filled with seawater (34 parts per thousand salinity) from the dolphin's home pool. The level of seawater in the container was sufficient to keep the water surface just above the level of the dolphin's eyes. During the experiment, seawater was poured or sponged over the dorsal portion of the animal to prevent drying. The catheter tubing was connected through a port in the dolphin transport container and inserted into a urine collection vessel.

2.2.1. Sample collection

Blood and urine sample collections were conducted every hour over a desired 24–25 h. In some cases, issues related to catheter placement or animal comfort did not enable studies to persist for the full 24–25 h; the shortest studies ($n = 2$) lasted 16–17 h. For blood collection, the dolphins flukes were carefully lifted free of the water for obtaining blood samples of approximately 20 ml using a sterile 20 gauge disposable hypodermic needle. At hourly intervals, the urine collection vessel was emptied, measured, and an aliquot saved in a standard urinalysis vial.

2.2.2. Blood and urine diagnostics

Study plasma and urine variables included urea (mg/dl), sodium (mEq/L), chloride (mEq/L), potassium (mEq/L), and osmolality (mOsm/kg). Plasma and urine glucose (mg/dl) were measured during the fasting studies and the study involving seawater with protein ingestion. Specimens were stored at 4 °C. Periodically, plasma was collected from each heparinized blood tube after cells had settled. Plasma and urine were transported to a clinical laboratory experienced with dolphin specimens (Bioscience Laboratories, Van Nuys, California) for analysis. The following methodologies were employed: plasma urea, Autoanalyzer (Technicon SMA-12, Technicon Corp. Ardaley, NY); urine urea, modified urease and Berthelot method; sodium and potassium, flame photometry (Instrumentation Laboratory, Inc., Waterman, MS.); chloride, Buchler-Cotlove chloridometer (Buchler Instruments, Inc., Fort Lee, NJ). Urine and plasma osmolality were measured by freezing point

Table 1
Descriptions of nine feeding studies among six adult bottlenose dolphins (*Tursiops truncatus*).

Study number	Study type	Total hours	Study date	Animal ID	Plasma samples (#)	Urine samples (#)
1	Fasting	16	01 May	A	16	15
2	Fasting	23	25 November	B	23	23
3	10% Dextrose in ionosol G (3 L)	17	01 May	C	13	14
4	10% Dextrose in ionosol G (2 L)	25	24 September	D	17	17
3	Mackerel (10 lb)	24	13 March	E	15	24
6	Mackerel (11 lb)	24	13 March	F	16	26
7	Mackerel: (10 lb)	23	16 October	F	16	26
8	Mackerel: (10 lb)	24	26 November	F	16	26
9	Mackerel: (12 lb)	23	25 September	B	17	15