

California State University, San Bernardino

CSUSB ScholarWorks

Theses Digitization Project

John M. Pfau Library

1986

The effect of intraperitoneally administered thyroxine, triiodothyronine and iopanoic acid on the in vivo and in vitro oxygen consumption rates of normal (C57BL/KsJ DB/M) and diabetic (C57BL/KsJ DB/DB) mice

A. Kay. Kalousek

Follow this and additional works at: <https://scholarworks.lib.csusb.edu/etd-project>



Part of the [Endocrinology Commons](#), and the [Endocrinology, Diabetes, and Metabolism Commons](#)

Recommended Citation

Kalousek, A. Kay., "The effect of intraperitoneally administered thyroxine, triiodothyronine and iopanoic acid on the in vivo and in vitro oxygen consumption rates of normal (C57BL/KsJ DB/M) and diabetic (C57BL/KsJ DB/DB) mice" (1986). *Theses Digitization Project*. 363.

<https://scholarworks.lib.csusb.edu/etd-project/363>

This Thesis is brought to you for free and open access by the John M. Pfau Library at CSUSB ScholarWorks. It has been accepted for inclusion in Theses Digitization Project by an authorized administrator of CSUSB ScholarWorks. For more information, please contact scholarworks@csusb.edu.

THE EFFECT OF INTRAPERITONEALLY ADMINISTERED THYROXINE,
TRIIDOTHYRONINE AND IOPANOIC ACID ON THE IN VIVO AND
IN VITRO OXYGEN CONSUMPTION RATES OF NORMAL
(C57BL/KsJ DB/M) AND DIABETIC (C57BL/KsJ DB/DB) MICE

A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
in
Biology


by
A. Kay Kalousek
April 1986

THE EFFECT OF INTRAPERITONEALLY ADMINISTERED THYROXINE,
TRIIDOTHYRONINE AND IOPANOIC ACID ON THE IN VIVO AND
IN VITRO OXYGEN CONSUMPTION RATES OF NORMAL
(C57BL/KsJ DB/M) AND DIABETIC (C57BL/KsJ DB/DB) MICE

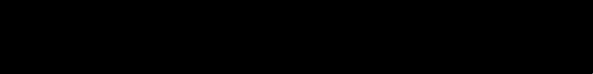
A Thesis
Presented to the
Faculty of
California State University,
San Bernardino

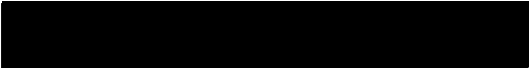
by
A. Kay Kalousek

Approved by:


Richard Fehn, Major Professor

4/8/06
Date


Dalton Harrington, Committee Member


Ruth Wilson, Chair, Biology

ABSTRACT

Oxygen consumption rates were measured in vivo and in vitro using 20+ week old genetically diabetic mice (C57BL/KsJ DB/DB). In one experiment, the in vitro oxygen consumption rate was measured at incubation temperatures of 35 and 37 degrees Celcius in order to replicate the average in vivo temperatures of diabetic and normal mice respectively. It was found that the incubation temperature did not affect the oxygen consumption rates in either group ($P>0.05$). Another group of experiments measured the effect that injections of iopanoic acid (IOP), IOP+T₃, or IOP+T₄ had on oxygen consumption. It was found in vivo that the mean oxygen consumption of normal mice was highest in the group that received IOP+T₃ when compared to all other normal or diabetic groups. However, the mean oxygen consumption of diabetic mice receiving IOP+T₄ was higher than all other diabetic groups, but was never as high as any of the normal groups. After the in vivo oxygen consumption measurements were made, the animals were sacrificed and oxygen consumption rates were measured in vitro using liver tissue homogenate. It was found that both normal and diabetic mice receiving IOP+T₄ had higher mean oxygen consumption rates than animals receiving IOP+T₃ or IOP alone. It was also found that diabetic mice receiving IOP+T₄ had higher mean oxygen consumption rates than normal animals receiving IOP or IOP+T₃. The

discussion suggests that these findings may help explain the obesity and some of the metabolic derangements found in diabetic mice and poses ideas for future research.

ACKNOWLEDGEMENTS

I sincerely thank my graduate committee, Dr. Richard Fehn, Dr. Ruth Wilson and Dr. Dalton Harrington for their review of my thesis and their helpful suggestions for its completion. Special thanks to my major professor, Dr. Richard Fehn for his many hours of encouragement, support and technical assistance. His friendship has been an invaluable source of pleasure and his guidance has helped to change the course of my life and for this I will always be grateful.

Thanks also to Dwight Gallo for his assistance in obtaining equipment and materials, as well as for his advice in overcoming some of the technical problems in the experiment.

Thanks to Dr. Diance Halpern for her review of and advice concerning the statistical aspects of the experiment.

My family has always been an ever-present source of support and encouragement for which I am sincerely grateful.

Finally, particular thanks to Jean Kayano for caring emotional support when everything was going wrong, for endless hours of glassware washing, frequent late nights and superb assistance with typing and artwork.

TABLE OF CONTENTS

List of Tables	vii
List of Figures.vii
Introduction.	1
Materials and Methods.9
Results.	15
Discussion.21
References.27

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. A comparison of the <u>in vivo</u> oxygen consumption rates ($\text{mm}^3 \text{O}_2 \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) of normal(+) and diabetic(db) mice injected intraperitoneally with saline vehicle, iopanoic acid (IOP), IOP+T ₃ or IOP+T ₄ .	16
2. A comparison of the <u>in vitro</u> oxygen consumption rates ($\mu\text{l O}_2 \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) of normal(+) and diabetic(db) mice injected intraperitoneally with saline vehicle, iopanoic acid (IOP), IOP+T ₃ or IOP+T ₄ .	18
3. RIA analysis of serum concentrations of T ₃ or T ₄ in normal(+) and diabetic(db) mice injected intraperitoneally with saline vehicle, iopanoic acid (IOP), IOP+T ₃ or IOP+T ₄ .	19

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. A small animal respirometer for the <u>in vivo</u> measurement of oxygen consumption by mice.	11

INTRODUCTION

Diabetes (db) is a mutation which occurred in the C57BL/KsJ inbred mouse strain (Hummel et al., 1966; Coleman and Hummel, 1967). The symptoms of the diabetic syndrome exhibited by these animals include obesity, hyperglycemia and hyperinsulinemia (Coleman and Hummel, 1967; Wyse and Dulin, 1970). Other symptoms such as polyuria, polydipsia, polyphagia and glycosuria occur when blood glucose levels reach 250-300 mg/100 ml (Hummel et al., 1966). The obesity, hyperglycemia, and hyperinsulinemia that occur in this mouse are similar to the metabolic disturbances that characterize human non-insulin dependent diabetes mellitus (NIDDM) (Hummel et al., 1966; Coleman and Hummel, 1967). For this reason, the diabetic mouse has been advanced as an animal model for the human diabetic syndrome.

Numerous studies have endeavored to characterize the physiological and biochemical disturbances that contribute to the diabetic syndrome in this mouse. Early work by Coleman and Hummel (1967) demonstrated that in the early stage of the disease there was an increase in plasma insulin and a higher than normal rate of lipogenesis, gluconeogenesis and glucose oxidation. They noted that despite high circulating levels of insulin, the animals remained hyperglycemic suggesting a defect in the action of insulin on peripheral tissue. Coleman and Hummel (1967) noted that after 12 weeks of age most of the animals

exhibited decreased levels of circulating insulin and decreased rates of glucose oxidation, but that the rate of gluconeogenesis remained high. These results were confirmed by Wyse and Dulin (1970) who also noted that the resultant chronic hyperglycemia ultimately led to pancreatic beta cell exhaustion which resulted in lethal diabetes in older animals.

Hepatic carbohydrate metabolism in diabetic mice was studied by Chan et al. (1975). These workers reported that after 8 weeks of age there was a three-fold increase in the liver glycogen content in the livers of diabetic animals and that glycogen turnover was higher than normal. These authors also noted that despite the prominent hyperglycemia and hyperinsulinemia found in these mice there was a paradoxical elevation in the activity of glycolytic and gluconeogenic enzymes. Since an elevated insulin level is normally antagonistic to the mechanisms that raise blood sugar, they proposed that the chronic hyperglycemia could be explained either by insulin resistance or an enhanced secretion of glucagon that was not suppressed by high glucose levels. One other finding reported by Chan et al. (1975) was that the liver of diabetic mice was, on the average, twice as large as that of normal mice. This enlargement was found to be primarily due to increased fat deposition since the DNA content of the tissue was only 70% of normal liver tissue indicating reduced non-adipocyte

cellularity in diabetic liver tissue (Chan et al., 1975).

In addition to these biochemical investigations, a metabolic study performed by Trayhurn (1979) showed that the body temperature of the diabetic mouse was from 1.1 to 4.5 degrees Celcius lower than that of normal animals maintained at ambient temperatures between 10 and 33 degrees. In addition, diabetic mice that were equilibrated in temperatures ranging from 4 to 30 degrees exhibited resting metabolic rates, based upon oxygen consumption, that were approximately 25% below those of non-diabetic mice.

Despite extensive study, no adequate explanation for the etiology of the diabetic syndrome has been substantiated. It has been proposed that the disease may be due to a general hormonal dysfunction (Coleman and Hummel, 1967). Alternately, the observed biochemical and endocrine abnormalities in diabetic mice may be symptoms of a more fundamental problem. Recently Fehn (1984) has hypothesized that the causative factor may lie in a basic physiological element that has a wide range of influence over the homeostatic mechanisms of the body. His studies have demonstrated a disturbance in the serum concentrations of the thyroid hormones 3,5,3' Triiodothyronine (T_3) and Thyroxine (T_4) which coincides with the onset of obesity, hyperglycemia, and hyperinsulinemia in diabetic mice. Fehn (1984) observed that nonfasting T_4 levels were depressed, T_3

levels were elevated, and the ratio of T_3 to T_4 was greatly increased. He proposed that since thyroid hormones mediate a wide variety of metabolic processes, the etiology of the diabetic state may be linked to abnormalities in the response of diabetic mice to thyroid hormones.

Thyroid hormones are synthesized by the follicular cells of the thyroid gland. These cells are able to take up iodine and transport it to the luminal surface of the cell where it is converted by a peroxidase to iodide (DeGroot et al., 1984; Hadley, 1984). This iodide is then covalently complexed to the tyrosyl residues of thyroglobin (TG), a protein which is synthesized by the follicular cells (DeGroot et al., 1984; Hadley, 1984). Tyrosyl residues are able to complex with either one or two iodides to form monoiodotyrosine (MIT) or diiodotyrosine (DIT) residues respectively, which can be oxidatively coupled to form T_3 or T_4 (DeGroot et al., 1984; Hadley, 1984). Stored MIT, DIT, T_3 , and T_4 complexed to TG make up the colloid of the follicular lumen (Hadley, 1984).

Although the primary form of thyroid hormone secreted by the follicular cells is T_4 , it is thought that T_3 plays the dominant role in effecting biological activity (Hadley, 1984). There is evidence, however, that points to a role for T_4 in at least some cellular processes (Larsen and Frumess, 1977; DeGroot et al., 1984). Triiodothyronine may also be produced from T_4 in peripheral tissues such as

liver and kidney through the action of the enzyme 5' monodeiodinase which removes the iodide from the 5' carbon of the diphenyl ring structure of the hormone (Chopra, 1977). The action of this enzyme can be blocked by several drugs including iopanoic acid (IOP) (Blum et al., 1974; Burgi et al., 1976; Obregon et al., 1980). It has been reported by Fehn (1984) that administration of IOP to diabetic mice normalizes the serum T₃ and T₄ levels by blocking the action of the monodeiodinase enzyme.

Thyroid hormones are involved in a wide variety of biological functions in homeothermic animals including stimulation of metabolic rate (Barker, 1951; Ismail-Beigi and Edelman, 1970; Oppenheimer, 1979; Sestoft, 1980; DeGroot et al., 1984; Muller and Seitz, 1984), regulation of growth and development (Oppenheimer, 1979; DeGroot et al., 1984; Hadley, 1984), thermogenesis (Edelman and Ismail-Beigi, 1975; Hadley, 1984; Muller and Seitz, 1984), irritability of the nervous system and increased cardiac output (De Groot et al., 1984). Administration of thyroid hormones stimulates oxygen consumption at both the whole body level and the cellular level (DeGroot et al., 1984). In a review of the available literature, Sestoft (1980) noted that the activities of a number of respiratory enzymes are increased under the influence of thyroid hormones. It has been hypothesized that this metabolic effect is due to one or several factors including

stimulation of protein synthesis (Buchanan et al., 1971; Sterling, 1979; DeGroot et al., 1984), stimulation of active sodium transport (Ismail-Beigi and Edelman, 1970; Ismail-Beigi and Edelman, 1971; Edelman and Ismail-Beigi, 1975; DeGroot et al., 1984); and stimulation of oxidative phosphorylation (Tata et al., 1963; Babior et al., 1973; DeGroot, 1979; Shears, 1980; Sterling, 1979; DeGroot et al., 1984; Sterling et al., 1984).

The mechanism of action for thyroid hormone bears similarities to that of the steroid hormones since both mediate their effects at the level of the nuclear DNA. However, the interaction of T_3 with cytosolic receptor proteins is significantly different than the interaction of a steroid with its receptors. It appears that cytosolic receptor- T_3 complexes do not bind directly to the chromatin, and therefore are not essential for hormone action (Hadley, 1984; Morgan, 1984). Once T_3 enters the nuclear envelope, it binds to a non-histone binding protein on the chromatin. Because the action of thyroid hormone is at least partially dependent upon transcriptional and translational events at the level of the genome there is a delay of about 24-48 hours before whole body measurements of thyroid hormone effects can take place.

Although the administration of T_3 and T_4 in vivo causes a concomitant increase in thermogenesis and oxygen consumption in homeotherms (DeGroot et al., 1984),

paradoxically, diabetic mice have higher than normal levels of serum T_3 (Fehn, 1984) yet lower than normal body temperatures and metabolic rates (Trayhurn, 1979). This suggests that although the animals appear to be hyperthyroid in the absolute sense, they are functionally hypothyroid, possibly due to a peripheral resistance to the action of thyroid hormone or a reduction in free T_3 which possibly results from the presence of T_3 -binding serum proteins (Kaplan et al., 1985).

Data from a prior study performed by this author (unpublished study, 1984) demonstrated greater in vitro oxygen consumption rates in liver homogenates from diabetic than in normal mice. These in vitro results seemed unusual in light of the study by Trayhurn (1979) which showed a lower than normal in vivo metabolic rate for diabetic mice. Since diabetic mice have a lower body temperature than normal mice, it was suspected that the elevated in vitro oxygen consumption rate might be due to an in vitro incubation temperature which was abnormal for the enzymatic systems of diabetic mice. Therefore, in the present study, oxygen consumption rates in diabetic and normal mice were measured in vitro using incubation temperatures of both 35 and 37 degrees Celcius, the average body temperature of diabetic and normal mice respectively. The remainder of this study focused on the effects of injections of iopanoic acid and thyroid hormones on the in vitro and in vivo

oxygen consumption rates of diabetic and normal mice.

Since iopanoic acid blocks the conversion of T_4 to T_3 , this study assessed the relative responsiveness of diabetic mice to the action of each hormone.

MATERIALS AND METHODS

Experimental Animals

Homozygous diabetic (db/db) and heterozygous normal (db/m) mice were obtained from the C57BL/KsJ breeding stock of Dr. Richard Fehn. This stock originated from a colony established at the Jackson Laboratory (Bar Harbor, Maine). The animals were housed at 23 degrees Celcius and 40-60% humidity in a Scherer environmental chamber. All animals were maintained on a lighting cycle of 14L:10D. Purina Rat Chow and water was available ad libitum for all animals.

Iopanoic Acid (IOP) and Thyroid Hormone Injection

Iopanoic acid (Telepaque, Winthrop Laboratories) was prepared immediately before use by sonicating 250 mg of the drug with 12.5 ml 0.1M NaOH. The solution was titrated with 0.1M HCl to an approximate pH of 7.8 and then diluted with distilled water to a volume of 25 ml (final concentration: 10 mg/ml) and protected from light.

Iopanoic acid was injected intraperitoneally using a volume of 0.5 ml/100g body weight (5.0 mg/100g body weight). The drug was administered within 25 minutes of preparation. Control animals received injections of saline vehicle prepared as above without iopanoic acid.

Solutions of thyroxine (T_4) and 3,5,3' triiodothyronine (T_3) (Sigma) were prepared daily by dissolving 0.4 mg/ml T_4 or 0.1 mg/ml T_3 in 2.0 and 0.5 mM

NaOH respectively. Experimental animals were injected intraperitoneally with either solution using a volume of 0.01 ml/g body weight.

Experimental animals (20+ weeks of age) were divided into four groups: saline vehicle, IOP, IOP+T₃, and IOP+T₄, each containing three diabetic and three normal mice. The treated groups were each administered a priming dose of IOP on the first day of the experiment. This was followed on day two by a second injection containing either IOP, T₃, or T₄. Blood samples were drawn and animals sacrificed on day three.

Measurement of Oxygen Consumption In Vivo

Four whole-body volumetric respirometers were constructed according to the method outlined by Smothers (1966). The apparatus (figure 1) was modified to delete the oxygen chamber so that oxygen could be introduced directly into the animal chamber with a 50 ml glass syringe. Leakage from the syringe was prevented with a three-way stopcock. In order to minimize oxygen loss between the manometer and the chambers, rubber tubing was replaced with glass tubing. Commercial 24 oz. glass food jars were used for both the animal chamber and compensating chamber. Freshly prepared 10% KOH was used as a CO₂ absorbent in the animal chamber.

Prior to the start of the experiment, one animal was

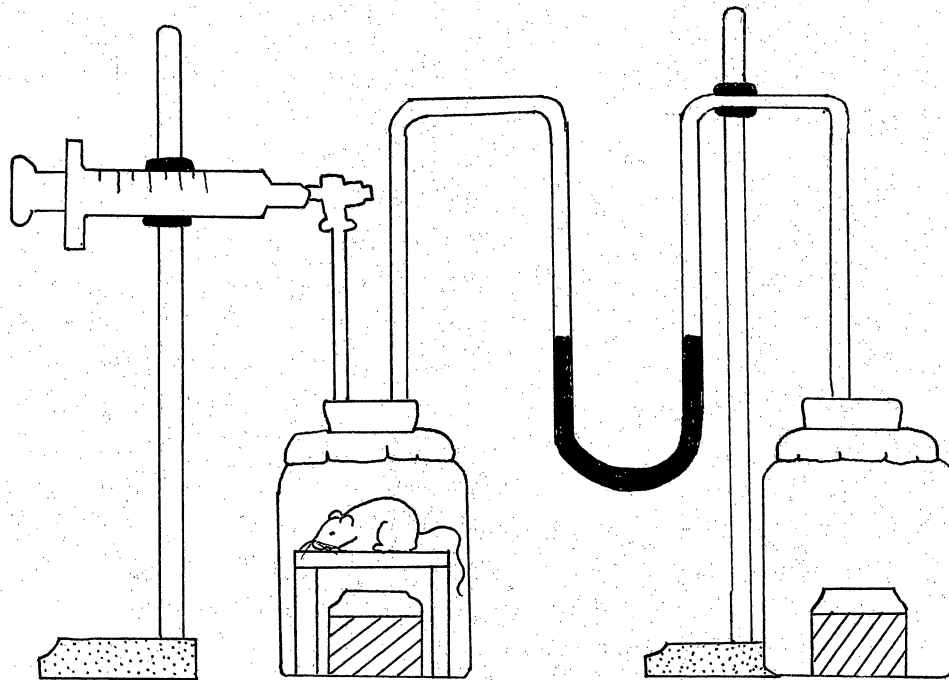


Figure 1. A small animal respirometer for the in vivo measurement of oxygen consumption by mice.

placed in each chamber and the Brodie's solution in each manometer was equalized between jars. The jars were then sealed and injected with 5.0 ml of oxygen. The addition of the oxygen resulted in a displacement of the Brodie's solution within the manometer. Oxygen consumption rates were based on the time necessary for the manometric fluid to return to the original level. Measurements were taken over a 30 minute period. All experiments were performed within the environmental chamber in order to avoid temperature fluctuations. Measurements were recorded between 0830 and 0900 hours.

Blood Collection

Blood was obtained by cardiac puncture using unheparinized 25 gauge needles and 1.0 ml syringes. Samples were allowed to clot at room temperature for one hour before centrifugation at 1500g for 15 minutes. Serum was pipetted into 1.5 ml eppendorf tubes and stored at -20 degrees Celcius until assayed. All collections were performed between 0900 and 1030 hours.

Preparation of Liver Homogenate

Animals were sacrificed by cervical subluxation immediately following blood collection and the whole liver was removed. The liver tissue was weighed and placed in a Pyrex mortar tube to which nine times (vol/wt) the tissue

weight of 10% glucose in 10mM phosphate buffer, pH 7.5, was added. The tissue was thoroughly homogenized using a Pyrex pestle driven by a Con-torque motor. The homogenate was filtered through four layers of gauze and stored in a glass beaker placed in an ice bath until assayed.

Dry weight of the liver homogenate was determined by pipetting 1.0 ml homogenate into planchets that were weighed before and after drying for 24 hours at 100 degrees Celcius.

Measurement of Oxygen Consumption In Vitro

Oxygen consumption was measured using a Gilson Differential Respirometer. The system was modified so that each channel had an individual balance flask joined by Tygon tubing to a bivalve digital manometer which was similarly connected to the reaction flask.

Prior to the start of the experiment, 0.2 ml 10% KOH was added as a CO₂ absorbent to the center well of each reaction flask. Liver homogenate (2.0 ml) was then pipetted into the flask and a filter paper wick (Whatman #40) was inserted into the center well. Distilled water (2.0 ml) was added to each balance flask to maintain vapor pressure and compensate for atmospheric pressure changes during the course of the experiment. Each flask was attached to a ground glass holder and lowered into a 37 degree Celcius water bath. Flasks were gassed with oxygen

while shaking for 10 minutes.

The experiment was initiated when the stopcock between the reaction and balance sides of the manometer was closed. Readings of the manometric fluid displacement due to tissue oxygen uptake were taken at 10-minute intervals for a period of 1.5 to 2.0 hours. Each manometer was calibrated with a Gilmont calibrator at the end of the experiment. Each treatment group contained 3 animals and each liver homogenate was assayed in triplicate.

Serum Hormone Level Assay

Serum T_3 and T_4 concentrations were determined using Amerlex radioimmunoassay kits (Amersham Corporation) according to their standard protocol.

Analysis of Data

Results of each experiment were evaluated by Student's t-test.

RESULTS

Effect of Temperature on In Vitro Oxygen Consumption

The mean respiration rates at standard conditions of temperature and pressure of pooled diabetic or normal mouse liver homogenates incubated at 37 degrees Celcius were 36.45 ± 2.66 and $36.09 \pm 1.87 \mu\text{l O}_2 \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ respectively. Samples taken from the same diabetic and normal liver homogenate pools and incubated at 35 degrees Celcius showed standard respiration rates of 40.03 ± 2.37 and $38.44 \pm 8.02 \mu\text{l O}_2 \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ respectively. T-test analysis of respiration rates showed no significant differences between any of the groups ($P > 0.05$).

Influence of IOP+T₃ or T₄ on In Vivo Oxygen Consumption

The results of this study are summarized in Table 1. In normal mice, the mean respiration rates between animals receiving saline vehicle, IOP, and IOP+T₄ were similar ($P > 0.05$). However, the mean oxygen consumption of normal mice that received IOP+T₃ was higher than each of these groups ($P < 0.01$). In contrast, the mean respiration rates between diabetic mice receiving saline vehicle, IOP, and IOP+T₃ were similar ($P > 0.05$), whereas the mean oxygen consumption of diabetic mice that received IOP+T₄ was higher than each of these groups ($P < 0.05$). In all treatments, the mean respiration rates of the diabetic mice were lower than any treatment of the normal mice.

Table 1. A comparison of the in vivo oxygen consumption rates ($\text{mm}^3 \text{O}_2 \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) of normal(+) and diabetic(db) mice injected intraperitoneally with saline vehicle, iopanoic acid (IOP), IOP+T₃ or IOP+T₄.

T-tests Between Groups (d.f.=4)

<u>Normal(+):</u>	+/veh	+/IOP	+/IOP+T ₃	+/IOP+T ₄	db/veh	db/IOP	db/IOP+T ₃	db/IOP+T ₄
Vehicle 4853.5± 90.0	-	ns	***	ns	***	***	***	***
IOP 5311.3± 421.9	ns	-	***	ns	***	***	***	**
IOP+T ₃ 6778.9± 232.4	***	***	-	***	***	***	***	***
IOP+T ₄ 4827.8± 514.4	ns	ns	***	-	**	**	***	*
<u>Diabetic(db):</u>								
Vehicle 3423.2± 143.1	***	***	***	**	-	ns	ns	***
IOP 3423.2± 286.8	***	***	***	**	ns	-	ns	**
IOP+T ₃ 3325.3± 69.2	***	***	***	***	ns	ns	-	***
IOP+T ₄ 4174.9± 136.5	***	**	***	*	***	**	***	-

Values are mean ± S.D.
(n=3)

* = $p \leq 0.1$ 2.13
 ** = $p \leq 0.05$ 2.78
 *** = $p \leq 0.01$ 4.60
 ns = non-significant

Influence of IOP+T₃ or T₄ on In Vitro Oxygen Consumption

Table 2 summarizes the mean in vitro respiration rates for liver homogenates of normal and diabetic mice receiving various treatments. In normal mice, the in vitro oxygen consumption rates were similar between saline vehicle and IOP+T₃ injected animals (P>0.05). The mean respiration rate of normal animals injected with IOP alone was lower than animals injected either with saline vehicle or IOP+T₃ (P<0.05), whereas the mean oxygen consumption rate for mice injected with IOP+T₄ was higher than the other three groups (P<0.05). Diabetic mice injected with saline vehicle had mean respiration rates that were lower than those animals injected with IOP and IOP+T₄ (P<0.1). Diabetic mice injected with IOP+T₄ had a higher mean oxygen consumption rate than those injected with saline vehicle (P<0.05). Diabetic mice injected with IOP and IOP+T₄ had higher mean respiration rates than normal animals injected with saline vehicle, IOP or IOP+T₃.

Assay of Serum Thyroid Hormones

The results of RIA analysis of serum thyroid hormone levels in diabetic and normal animals receiving various treatments is summarized in Table 3. There was no difference between the serum T₃ and T₄ concentrations of diabetic and normal mice injected with saline vehicle or IOP alone (P>0.05). However, diabetic mice which received

Table 2. A comparison of the in vitro oxygen consumption rates ($\mu\text{l O}_2 \cdot \text{g}^{-1} \cdot \text{min}^{-1}$) of normal(+) and diabetic(db) mice injected intraperitoneally with saline vehicle, iopanoic acid (IOP), IOP+T₃ or IOP+T₄.

T-tests Between Groups (d.f.=4)

<u>Normal(+):</u>	+/veh	+/IOP	+/IOP+T ₃	+/IOP+T ₄	db/veh	db/IOP	db/IOP+T ₃	db/IOP+T ₄
Vehicle 33.93± 4.93	-	**	ns	**	ns	**	ns	***
IOP 25.57± 9.23	**	-	***	***	***	***	**	***
IOP+T ₃ 35.39± 3.95	ns	***	-	*	ns	*	ns	**
IOP+T ₄ 41.92± 8.87	**	***	*	-	ns	ns	ns	ns
<u>Diabetic(db):</u>								
Vehicle 36.11± 1.80	ns	***	ns	ns	-	*	ns	**
IOP 42.47± 10.50	**	***	*	ns	*	-	ns	ns
IOP+T ₃ 48.27± 29.30	ns	**	ns	ns	ns	ns	-	ns
IOP+T ₄ 49.14± 14.85	***	***	**	ns	**	ns	ns	-

Values are mean ± S.D.
(n=3)

* = $p \leq 0.1$ 1.75
 ** = $p \leq 0.05$ 2.12
 *** = $p \leq 0.01$ 2.92
 ns = non-significant

Table 3. RIA analysis of serum concentrations of T₃ or T₄ in normal(+) and diabetic(db) mice injected intraperitoneally with saline vehicle, iopanoic acid (IOP), IOP+T₃ or IOP+T₄.

Serum Levels of Thyroid Hormone:

	T ₃ (ng%)	T ₄ (µg%)
<u>Normal(+):</u>		
Vehicle	53.00±3.60	3.67±1.77
IOP	43.30±17.95	4.06±2.85
IOP+T ₃	1778.70±173.70	3.42±0.63
IOP+T ₄	654.00±200.23	70.59±32.91
<u>Diabetic(db):</u>		
Vehicle	50.30±3.78	1.92±0.63
IOP	60.00±16.52	3.87±0.82
IOP+T ₃	2129.67±354.86	3.54±0.86
IOP+T ₄	520.33±22.50	37.67±0.88

Values are mean ± S.D.
(n=3)

IOP showed an increase in serum concentrations of T_4 ($P < 0.05$). The highest serum concentrations of T_3 were found in the normal and diabetic mice receiving IOP+ T_3 ($P < 0.01$). The highest concentrations of serum T_4 were found in the normal and diabetic mice which received IOP+ T_4 ($P < 0.01$). There was also an increase from normal in the serum concentrations of T_3 in diabetic and nondiabetic mice administered IOP+ T_4 .

DISCUSSION

Data from an earlier experiment by this author (unpublished study, 1984) had demonstrated an in vitro oxygen consumption rate for the liver tissue homogenate of saline vehicle injected diabetic and normal mice of 55.6 ± 4.37 and $22.8 \pm 2.77 \mu\text{l O}_2 \cdot \text{g}^{-1} \cdot \text{min}^{-1}$ respectively at standard conditions. Both the diabetic and normal animals in this study were less than 12 weeks old. These data indicated that liver tissue in diabetic mice had a higher oxygen consumption rate than that of normal mice. This result was surprising as it had been expected that the metabolic rate of diabetic mice would be lower than normal, which could help to explain the obesity of the animals. However, since this study had been performed at 37 degrees Celcius, which is the normal body temperature for non-diabetic mice but elevated for diabetic mice, it was thought that the high oxygen consumption rate observed in diabetic mouse tissue homogenate might be an artifact of an abnormal incubation temperature. The present study demonstrates that there is no difference in the oxygen consumption rates of diabetic and normal mouse liver homogenates incubated at either 35 or 37 degrees Celcius. Therefore, incubation temperature was likely not responsible for the higher than normal oxygen uptake rates that were observed in 10 week old diabetic mice.

Although the previous study had demonstrated a higher

($P < 0.01$) oxygen consumption rate in diabetic mouse liver tissue, this was not replicated in the study using 20+ week old animals. Since measurements of oxygen consumption at 37 degrees Celcius were performed similarly in both experiments, other variables were considered to explain this discrepancy.

One explanation may lie in the ages of the diabetic animals that were used in the respective experiments. In the previous study, the diabetic mice were less than twelve weeks old. This would place them in the early stage of the disease (Coleman and Hummel, 1967; Wyse and Dulin, 1970) in which the processes of lipogenesis, gluconeogenesis and glucose oxidation are abnormally high. In contrast, the animals used in the present study were much older (20+ weeks old). It has been reported (Coleman and Hummel, 1967; Wyse and Dulin, 1970) that the metabolic condition of the diabetic mouse deteriorates with increasing age. These workers both demonstrated decreased glucose utilization and glucose oxidation in older diabetic mice. Since the present study was conducted using older animals, it is likely that the discrepancy observed in the oxygen consumption rates of the two experiments could be due to the effects of age upon the metabolic processes of diabetic mice.

Different responses were also seen in the in vivo and in vitro oxygen consumption rates of diabetic and normal

mice treated with thyroid hormones and iopanoic acid. As was mentioned above, it is thought that T_3 is the hormone primarily responsible for initiating metabolic responses such as increased oxygen consumption. It is also established that IOP blocks the conversion of T_4 to T_3 . Therefore, it was expected that the oxygen consumption rate of animals receiving injections of IOP might be reduced due to lower levels of T_3 . It was also expected that administration of IOP+ T_3 would raise the metabolic rate since IOP is not thought to have any effect on the action of T_3 . Injections of IOP+ T_4 would, however, reduce the oxygen consumption rate since the conversion of the injected T_4 as well as the endogenous T_4 would be blocked, thus preventing the formation of metabolically active T_3 .

In the in vivo oxygen consumption study, normal mice exhibited an increased respiration rate when given IOP+ T_3 ($P < 0.01$). There was no increase in the in vivo respiration rate of normal mice administered IOP+ T_4 ($P > 0.05$). Conversely, the in vivo oxygen consumption rate of diabetic mice given IOP+ T_3 was not increased while that of diabetic mice given IOP+ T_4 was higher than all other diabetic animal groups ($P < 0.05$).

The in vivo results were, however, not consistent with the in vitro liver homogenate oxygen consumption rates of diabetic and normal mice. In normal mice, IOP+ T_3 did not raise the oxygen uptake rate above normal ($P > 0.05$), but

administration of IOP+T₄ raised the respiration rate above all other normal groups. When IOP+T₄ was given to diabetic mice, the oxygen consumption rate was also raised above normal (P<0.05). However, the oxygen consumption rate of diabetic mice given IOP+T₄ was not higher than the diabetic animal groups given IOP and IOP+T₃.

There are two major discrepancies between results of the measurement of the in vivo and in vitro oxygen consumption rates in diabetic and normal mice. The first is that the in vivo oxygen uptake of normal mice is increased by administration of IOP+T₃, but in vitro an increase is noted only when IOP+T₄ is given. The diabetic mouse, on the other hand, exhibits increased oxygen consumption only with IOP+T₄ both in vivo and in vitro. Secondly, the in vivo oxygen consumption rate of all groups of normal mice are significantly higher than those of corresponding diabetic mice. However, the in vitro oxygen consumption rates of diabetic mice given various treatments are higher than several of the normal counterparts. In no case is the in vitro oxygen consumption rate of diabetic mice lower than that of the corresponding normal mice.

Two results in the RIA determination of serum thyroid hormone concentrations are of particular interest. The administration of IOP to diabetic mice caused an increase in the serum concentration of T₄ above the levels of saline vehicle injected diabetic mice (P<0.05). This result was

expected since IOP blocks the conversion of T_4 to T_3 and this would tend to raise serum concentrations of the hormone. It is not known, however, why this effect was not seen in similarly treated normal mice.

The other interesting finding is the increase in serum T_3 concentrations exhibited in both diabetic and normal animals which received IOP+ T_4 . Since IOP blocks the deiodination of T_4 to T_3 , it seems anomolous to find increased T_3 levels. It may be that the concentrations of T_4 administered to these mice were sufficiently high to overcome the blocking action of IOP, thus partially negating its effect. Another explanation lies in the nature of the radioimmunoassay. Amersham Company states in the instruction manual for the RIA kit that the antibody for T_3 has a 0.3% cross-reactivity with T_4 . Since T_4 concentrations exceed T_3 concentrations by more than one thousand-fold, part of the increase in T_3 levels in animals given IOP+ T_4 may be explained by this effect. It is possible that a combination of these two factors may contribute to the elevated T_3 levels. Contaminant T_3 in the T_4 reagent could also account for the increase.

In summary, in vitro oxygen consumption rates of diabetic mice are lower in older animals (20+ weeks) compared to 10 week old animals. The oxygen consumption response to IOP+ T_3 and IOP+ T_4 appears to be different in vivo and in vitro between normal mice and diabetic mice.

In vivo, normal mice are responsive to the action of T_3 while diabetic mice are more responsive to the action of T_4 or possibly to the deiodination of T_4 . Diabetic mice, however, have consistently lower oxygen consumption rates. In vitro, both normal and diabetic mouse liver homogenates show oxygen consumption rate increases only in the presence of T_4 . This may indicate a requirement for intracellular deiodination of T_4 to stimulate the increased metabolic response. Of greatest importance is the observation that diabetic animals exhibit significantly lower metabolic responses to T_3 compared to similarly treated normal animals. This suggests that the obesity associated with non-insulin dependent diabetes may be the result of tissue resistance to the action of T_3 . Further studies concentrating on age differences in the metabolic response to thyroid hormones and differences between the whole body and specific cellular responses to thyroid hormones in diabetic and normal mice will be necessary to clarify these issues.

REFERENCES

- Babior, M.B., S. Creagan, S.H. Ingbars and R.S. Kipnes (1973). Stimulation of Mitochondrial Adenosine Diphosphate Uptake by Thyroid Hormones. Proceedings of the National Academy of Science 70(1):98-102.
- Barker, S.B. (1951). Mechanism of Action of the Thyroid Hormone. Physiological Reviews 31(3):205-243.
- Blum, M., U. Weinberg, L. Shenkman and C.S. Hollander (1974). Hyperthyroidism After Iodinated Contrast Medium. The New England Journal of Medicine 291(1): 24-25.
- Buchanan, J.L., M.P. Primack and D.F. Tapley (1971). Effect of Inhibition of Mitochondrial Protein Synthesis In Vitro Upon Thyroxine Stimulation of Oxygen Consumption. Endocrinology 89:534-537.
- Burgi, H., C. Wimpfheimer, A. Burger, W. Zaunbauer, H. Rosler and T. Lemarchand-Beraud (1976). Changes of Circulating Thyroxine, Triiodothyronine and Reverse Triiodothyronine after Radiographic Contrast Agents. Journal of Clinical Endocrinology and Metabolism 43: 1203-1210.
- Chan, T.M., K.M. Young, N.J. Hutson, F.T. Brumley and J.H. Exton (1975). Hepatic Metabolism of Genetically Diabetic (db/db) mice. I. Carbohydrate Metabolism. American Journal of Physiology 229(6):1702-1712.
- Chopra, I.J. (1977). A Study of Extrathyroidal Conversion of Thyroxine (T₄) to 3,5,5' Triiodothyronine (T₃) In Vitro. Endocrinology 101:453-463.
- Coleman, D.L. and K.P. Hummel (1967). Studies with the Mutation, Diabetes, in the Mouse. Diabetologia 3:238-248.
- De Groot, L. (1979) Thyroid Hormone Action. In: Endocrinology. L. De Groot, gen ed. Grune and Stratton, Inc., New York. pp. 357-363.
- De Groot, L., J.B. Stanbury, P.R. Larson and S. Refetaff (1984). The Thyroid and its Diseases. John Wiley and Sons, New York. pp. 36-74.

- Edelman, I.S. and F. Ismail-Beigi (1975). Thyroid Thermogenesis and Active Sodium Transport. Recent Progress in Hormone Research 30:235-257.
- Fehn, R. (1984). Pituitary Thyroid Function in the C57BL/KsJ DB/DB Mouse. Doctoral Dissertation, University of Arizona.
- Hadley, M.E. (1984). Endocrinology. Prentice Hall Inc.: Englewood Cliffs, New Jersey. pp. 292-307.
- Hummel, P.K., M.M. Dickie and D.L. Coleman (1966). Diabetes, a New Mutation in the Mouse. Science 153: 1127-1128.
- Ismail-Beigi, F. and I.S. Edelman (1970). Mechanism of Thyroid Calorigenesis: Role of Active Sodium Transport. Proceedings of the National Academy of Science 67(2):1071-1078.
- Ismail-Beigi, F. and I.S. Edelman (1971). The Mechanism of the Calorigenic Action of Thyroid Hormone. Journal of General Physiology 57:710-722.
- Kalousek, A.K. (1984). In Vitro Cellular Respiration in Diabetic-Obese(db/db) and Normal(db/+) Mouse Liver: A Comparative Study. Unpublished study.
- Kaplan, M., J. Young, and E. Shaw. (1985). Abnormal Thyroid Hormone Binding to Serum Proteins in Ob/Ob and Db/Db Genetically Obese Mice. Endocrinology 117:1858-1863.
- Larsen, P.R. and R.D. Frumess (1977). Comparison of the Biological Effects of Thyroxine and Triiodothyronine in the Rat. Endocrinology 100:980-988.
- Morgan, G. (1984). Thyroid Hormone Binding in Rat Liver Cytosol. Journal of Endocrinology 103:265-271.
- Muller, M.J. and H.J. Seitz (1984). Thyroid Hormone Action on Intermediary Metabolism. Klinische Wochenschrift 62:11-18.
- Obregon, J., A. Pascual, J. Malloi, G. Morreale de Escobar and F. Escobar (1980). Evidence Against a Major Role of L-Thyroxine at the Pituitary Level: Studies in Rats Treated with Iopanoic Acid (Telepaque). Endocrinology 106:1827-1836.

- Oppenheimer, J.H. (1979). Thyroid Hormone Action at the Cellular Level. Science 203:971-979.
- Sestoft, L. (1980). Metabolic Aspects of the Calorigenic Effect of Thyroid Hormone in Mammals. Clinical Endocrinology 13:489-506.
- Shears, S.B. (1980). The Thyroid Glands and the Liver Mitochondrial Protonic Electrochemical Potential Difference: A Novel Hormone Action?. Journal of Theoretical Biology 82:1-13.
- Smothers, J.L. (1966). A Volumetric Respirometer for Small Animals. Journal of Applied Physiology 21(3): 1117-1118.
- Sterling, K. (1979). Thyroid Hormone Action at the Cell Level. The New England Journal of Medicine 300(3): 117-123.
- Sterling, K., G.A. Campbell, G.S. Taliadouros and E.A. Nunez (1984). Mitochondrial Binding of Triiodothyronine (T₃). Cell and Tissue Research 236:321-325.
- Tata, J.R., L. Ernster, O. Lindberg, E. Arrhenius, S. Petersen and R. Hedman (1963). The Action of Thyroid Hormones at the Cell Level. Biochemical Journal 86: 408-428.
- Trayhurn, P. (1979). Thermoregulation in the Diabetic Obese (db/db) Mouse. Pflugers Archiv 380:227-232.
- Wyse, B.M. and W.E. Dulin (1970). The Influence of Age and Dietary Conditions on Diabetes in the Db Mouse. Diabetologia 6:268-273.