Varying macronutrient ratios in the ketogenic diet for type 2 diabetes treatment in animals

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Abstract

My research aims to determine the best macronutrient ratio within the ketogenic diet that provides the best glycemic control of animals with Type II Diabetes Mellitus. Preliminary research shows a benefit in the use of the ketogenic diet as a form of diabetic management; however, the precise chemical composition of the diet as not yet been explored. Treating Type II Diabetes in animals is often costly, as pet owners must provide insulin injections to their pets 1-3 times a day. Adjusting a pet's diet may be a safer alternative form of treatment than insulin injections and other diabetic medications. To see the effects of this diet on the diabetes, glucose concentrations in the blood were measured using an over-the-counter glucometer and glycated albumin levels were determined by the use of an ELISA. Preliminary research results show unusual behavior in glucose measurements.

Introduction

- Type 2 diabetes mellitus (T2DM) characterized by dysregulation of carbohydrate, lipid and protein metabolism.
- Patients are unable to produce or respond to insulin properly, resulting in abnormally high blood sugar levels. Elevated sugar, or glucose, can have harmful effects in the body.
- Hyperinsulinemia will cause body to secrete an increasing amount of insulin hormone as a result of the high glucose levels, leading to insulin resistance.
- Insulin injections needed for diabetic patients to assist the pancreas's secretion of insulin. The pancreas cannot make enough insulin on its own, so insulin injections are administered as supplemented insulin.
- The pancreas cannot overcome the amount of insulin needed to reverse insulin resistance on its own, requiring another method.
- Research has already been done regarding the ketogenic diet for T2DM treatment, which demonstrates that strictly reducing carbohydrate intake and increasing fat and protein intake were significantly effective in improving glycemic control.
- Other research is focused more heavily on altering either protein or fat in the ketogenic diet or using consistent amounts of both macronutrients, this research aims to test the effects of manipulating both amounts of both protein and fats in the ketogenic diet to see which proportion of fat and protein will provide the best glycemic control.
- Goal is to find and provide a better route to reversing insulin resistance that could lead to improvements in diabetic management and control for animals battling T2DM.

Methodology

- Sixteen 6-week-old male rats were randomly split into a control, 100% fat/0% protein, 50% fat/50% protein, and 0% fat/0% protein groups.
- Each rat injected with a 40mg/kg dose of streptozotocin in Hank's Balanced Buffer. Injections performed twice, each one being one week apart.
- Diabetes confirmed using a urine dipstick and blood glucose measurements.
- Control group fed the standard rat chow, while the experimental groups were fed the basic nutrients needed for growth, supplemented with the homemade ketogenic diet for their energy needs.
- Fasting and post-prandial blood glucose measurements taken once per week via glucometer. All blood samples were acquired via the tail snip method.
- Blood samples collected and centrifuged after injections and during the last week of the study. The serum was stored in the freezer for the ELISA protocol until use.
- Once ready, samples were pulled out the day before the ELISA procedure to thaw.
- The microliters of serum present in each microfuge tube were estimated using a micropipette. If there was less than 40µL, DI water was added to make the total volume in each tube 50µL.
- ELISA performed and results acquired from concentrations and standard curve.

Results

Table 1. Data of average fasting and postprandial blood glucose and their respective standard deviations each week that data was recorded. (BG=blood glucose, PP=postprandial). Blood glucose was measured in mg/dL.

Group	Week	Avg. Fasting BG	Std. Dev. Fasting BG	Avg. PP BG	Std. Dev. PP BG
Control	1	122	13.3	N/A	N/A
	2	101	9.74	129	10
	3	100	8.42	123	28.8
	4	112	21.9	140	10.5
	6	112	9.46	129	10.5
	7	108	9.68	124	9.46
100% fat/0% protein	1	120	12.5	N/A	N/A
	2	125	10.3	128	21.7
	3	118	13.3	128	5.91
	4	135	12.2	137	14.4
	6	124	6.14	131	10.8
	7	118	9.33	126	14.8
50% fat/50% protein	1	102	6.98	165	40
	2	119	18.1	124	6.45
	3	116	18	125	10.2
	4	119	12.7	126	13.7
	6	126	15.4	127	7.62
	7	121	3.11	114	15.3
0% fat/100 % protein	1	107	5.38	130	19.8
	2	113	18.5	127	15.1
	3	94	9.29	113	8.85
	4	117	11.8	131	10.2
	6	109	5.94	132	9.4
	7	109	6.45	132	2.99

Figure 1. Standard curve for glycated albumin (GA) ELISA prepared via serial dilution of standard stock solution in standard diluent. The equation for the line of best fit is y=0.0025x-0.0273 with a correlation coefficient of 0.9409.

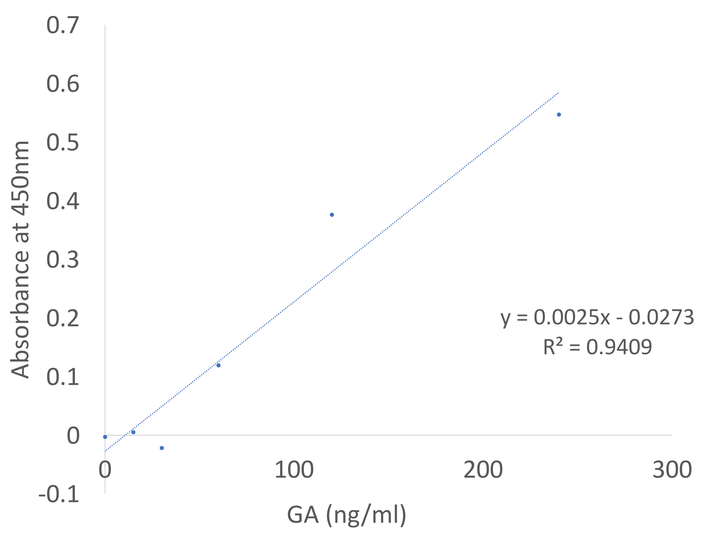

Table 2. Average and standard deviations of glycated albumin concentration (ng/mL) of each group during Week 1 and Week 8 of the study. Results of the GA ELISA are inconclusive due to very large standard deviations.

Group	Week 1 [GA]		Week 8 [GA]	
	Avg (ng/ml)	Std. Dev. (ng/ml)	Avg (ng/ml)	Std. Dev. (ng/ml)
Control	19.6	23.5	371.0	444.1
100% fat/0% protein	123.7	88.1	202.8	197.3
50% fat/50% protein	543.8	702.4	251.6	111.8
0% fat/100% protein	162.6	204.7	103.4	125.8

Discussion

The data shows that there was no significant change in either fasting or postprandial blood glucose measurements for any of the groups over the 7 weeks of the study. This could be due to the fact that the rats never truly reached a diabetic blood glucose concentration, but for time and resource reasons, the study was to go on with the values given. The GA ELISA had very high standard deviations for the concentrations of GA present in each of the samples. This means that this data was inconclusive, and nothing can be drawn from those values. This could be due to the dilutions performed for the insufficient-volumed samples.

Additional research can be done to expand upon this study by first ensuring diabetic states in each animal and a purer ketogenic diet than what was homemade with readily available vegetable oil and soy protein.

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References

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