Inquiry on Rate of Decline of Blood Glucose due to the Presence of a Potential Threshold

Research Question: Is there a threshold in blood glucose in which once below it, the rate of decline of blood glucose increases?

Subject of Essay: Biology
Supervisor Name: Gonca ESENDEMİR
Candidate Name: Kaan ÜNLÜ
Candidate Number: 001129-0039

Exam Session: May 2016
Word Count: 4180 (206 of which is data annotation in DCP)

ABSTRACT

This observation inquires about the presence of a threshold in blood glucose, when once above it, its rate of decline will change. The basis for argument comes from the laws of diffusion. Hence the following was questioned: **" Is there a threshold for blood glucose level in which once above it, its rate of decline increases?"** and it was hypothesized that **"where the starting blood glucose value is the independent variable and the change in blood glucose level and the rate of that change in set time are the dependent variables, it can be said that there is a certain threshold for blood glucose in which once above it, its decline rate will increase."**

In the observation were two voluntary diabetic subjects, one of them being me; living under a specific diet for five days, checking our blood glucose values frequently in predetermined intervals as trials. After the trials, I took the fasting periods' Δ blood glucose values (ΔG) and graphed the ones pointing to declines in blood glucose, seeing that ΔG increased with increasing initial blood glucose, regardless of whether any intervention took place when said value was encountered. Finally, to check whether found trend was meaningful in context, a paired samples t test was run with an hypothesized threshold of 130 mg/dl for the sake of statistics, which resulted in a (T<=t) one tail value of 0.00576, which was lower than the alpha value of 0.05, consequently rejecting the null hypothesis and confirming the disparities between the values, therefore also accepting the hypothesis: It can indeed be said that there is a threshold in blood glucose in which once above it, its rate of decline will increase, but since the test group consisted of only 2 samples, it's result is in no way ultimate.

Word Count: 296

TABLE OF CONTENTS

DESIGN

INTRODUCTION

As a simple carbohydrate, glucose is a primary nutrient for animal cells, which is chiefly stored as glycogen in the liver. As unlike cells, the brain is not capable of storing this glucose, an amount of glucose is left in the blood. Also, this blood glucose is needed for a possible emergency low cell energy condition in which these cells will be needing immediate energy supply.

Blood glucose fluctuation is a perfectly normal set of events that unfold anytime something is done to tilt the equilibrium of blood glucose. When such happens, blood glucose changes accordingly; decreasing via insulin when it is supplied with more (e.g. eating), and increasing via glucagon when cells start needing more nutrients (e.g. sports). Increasing the scale of fluctuations by extreme anabolism or catabolism (lower or higher values than the range of 70-140mg/dl) may and eventually do result in dizziness, vision impairment, lethargy, apprehension, trembling of bodily extremities and even nausea. Logic at this point dictates that **with increasing amount of nutrient need, more glucose from blood will be needed to be absorbed by the cells**; or conversely, **a larger amount of glucose supplied to the blood will result in bigger need for its distribution, thus increasing insulin secretion, and increasing the exposure time to first high then low blood glucose levels while doing so.**

What makes insulin so important is the role they have in the cells' intake of nutrients. A cell's main energy source is glucose, which can only pass through its membrane via insulin, which acts like the way a key does, opening the channels on the cell membrane that are wide enough for glucose to pass through and get in the cell, thus reducing blood glucose level and supplying cells with glucose. Then, by negative feedback, after enough transaction occurs, ideally, secretion stops, preventing the extinction of glucose in blood.

Insulin is secreted from the β cells in the Langerhanse Islets in the pancreas. When, due to a malfunction that's reasons are still unidentified, in the autoimmune system, the white blood cells recognise these cells as harmful and retaliate against them, eventually destroying them in a massive scale, or damaging them to such an amount that they are rendered practically useless. This case is called type 1 Diabetes Mellitus. Even though the reasons themselves are still obscure, it is known that there is about 6% chance of developing type 1 diabetes in a person with type 1 diabetic relatives, otherwise, this probability is about 0.5%[1].

Lack of insulin means that glucose stays in blood and cannot traverse the cell membrane, therefore being unable to supply the cells with energy, thus leaving the body tired and oversaturated with glucose alongside other symptoms and eventual wearing out of the organs in the body which ultimately results in death if not reverted to normal amounts; this is called

[1] http://www.nhs.uk/Conditions/Diabetes-type1/Pages/Causes.aspx

hyperglycaemia. Conversely, when insulin is injected into body from an outside source due to lack of secretion of it (Type 1 Diabetes), but in excess amounts, the blood is left without sufficient amount of glucose to supply the brain, and due to this absence, the brain ceases to work and the body enters a coma after a series of symptoms due to low blood glucose. This state of low blood glucose is called hypoglycaemia and is said to be an acute condition, as insulin rapidly unlocks channels in the cell membrane, causing a surge of glucose into the cells; whereas hyperglycaemia is said o be chronic, since glucose builds up relatively slowly in blood as meals enter the vessels while a certain amount of it is essentially aimed to be led to the liver to be stored.[2]

Interesting enough, by incidental observation as a Type 1 Diabetes case, I noticed that whenever my blood glucose is at values that may be considered or is close to a hypoglycaemia, it starts dropping considerably faster (maybe it's the result of positive feedback from hyperglycaemia); so, to see if this situation is incidental, somehow unique to me, or is not the case at all or not, I decided to test this phenomena on myself and someone I know to have Type 1 Diabetes, a female of about my size and biomass ratio measured by height and weight respectively, and their ratio in BMI[3]. This would give me an idea on whether or not what I'm thinking to be experiencing is feasible or not.

The observation is done to evaluate the rate of change of blood glucose of a body measured bymg/dl during its decline to see if there is a certain threshold in which the cells that are supplied blood glucose start absorbing such glucose at a faster rate, resulting in a hypoglycaemia when not treated. One speculation prior to the observations could be that the more a body's cells need nutrition (thus a case of high blood glucose), the more the insulin in blood that is used and the more the blood glucose declines, which in turn results in the host being moreencouraged to eat, which again stimulates the usage of left insulin in blood (the "left" insulin could be anything ranging fromminor remnants during a long period of fasting to accumulations due to lipohypertrophy to doses of newly injected insulin), resulting in an abrupt hypoglycaemia due to extensive feedback on a case of hyperglycaemia[4].Hence,the research questions go as following:**Is there a threshold for blood glucose level in which onceabove it, its rate of decline increases?**

In a body where there's no insulin secretion whatsoever and the body is given insulin from a regular external source at regular intervals and by regular amount and type of nutrition while body metabolism is kept constant by a regular amount of mental and physical stress and no diseases emerging, the hypothesis go as following:**"Where the starting blood glucose value is the independent variable and the change in blood glucose level and the rate of that change in set time are the dependent variables, it can be said that there is a certain threshold for blood glucose in which once above it, its decline rate will increase.**

[2] http://www.healthcentral.com/encyclopedia/hc/hypoglycemia---hyperglycemia-3168893/
[3]The weight of nations: an estimation of adult
human biomass, by Sarah Catherine Walpole, David Prieto-Merino, Phil Edwards, John Cleland, Gretchen Stevens and Ian Roberts, BMC Public health article
[4] http://www.vivo.colostate.edu/hbooks/pathphys/endocrine/pancreas/insulin.html

METHOD DEVELOPMENT

It should be noted that the whole process was undertaken by the free will of the subjects and under a medical doctor's full consent.[5]

This observation was planned to evaluate the rate of change of blood glucose of a body measured by mg/dl during its decline to see if there is a certain threshold in which the cells that are supplied blood glucose start absorbing such glucose at a faster rate, resulting in a hypoglycaemia when not treated.

Therefore,with an independent variable of "different starting blood glucose levels", the two dependent variables,change and the rate of that change of the subjects' blood glucose (in mg/dl) can be introduced, in which through this, the presence of a said threshold isinquired.

Besides that, the following factors were kept constant as much as possible to let the inquiry continue with a minimum amount of problems;

Apart from the fact that both subjects are Turkish humans that have lived in the same environment for more than the last 11 years of their life in the same school, the most essential aspect of these subjects is that they both have a case of Type 1 Diabetes. That is because without the case of **not** being able to secrete insulin, it becomes virtually impossible to ascertain whether or not such a threshold is present because the blood glucose level is automatically regulated by the body and thus which drop or rise in blood glucose happened by what reason remains unknown. To increase the reliability of the observations, both subjects recorded in the same dates in which all these days were school days; this also made the mealtimes more consistent. Thus, overall, the amount of meals the intervals at which they checked their blood glucose values were constant.[6]

Moreover, while choosing the female subject,(as the male subject is me) her biomass measured via mass and size via height was aimed to be as similar to the male subject's as possible for them to have a close BMI (basically size per unit weight) value between these specimens.[7]

The specimens were given a certain standard diet that complies with their lives and necessities to be followed during the observation period, that consisted of adequate amounts of carbohydrates in which that adequate amount was discussed and arrived at an agreement between the subjects themselves and with as low fat values as possible, completely avoiding it wherever possible. Nutrients taken as an immediate reaction to low blood glucose were agreed to work on the same

[5] Check Appendix-1
[6] A model for the daily schedule is available at appendix-3, but it should be noted that this program just serves as a guide to understanding how the schedule works and is thus applied according to the outside conditions with appropriate modifications.
[7] Check DCP section for details

low-fat principle, with eating factory products such as chocolate bars that consisted of less than 25% fat and where such is not possible, with lumps of sugar[8].

> The apparatus work as following in principle: "Current Glucometers use test strips containing glucose oxidase, an enzyme that reacts to glucose in the blood droplet, and an interface to an electrode inside the meter. When the strip is inserted into the meter, the flux of the glucose reaction generates an electrical signal. The Glucometer is calibrated so the number appearing in its digital readout corresponds to the strength of the electrical current: The more glucose in the sample, the higher the number."[9]

Due to economical reasons, no new apparatus was bought for the measurements, instead, subjects used their own equipment.[10]

As long as the observation proceeded, it was found appropriate, due to both for the subjects to be able check their blood glucose at break times while in school and to have a tighter control over blood glucose, especially right after insulin injection, that the meal should be finished preferably below 30 minutes and blood glucose checkpoints should be 90 minutes after rapid insulin injection, another 45 minutes after that, and every 90 minutes after that till the next injection.

Though it could not be forced, the subjects were asked to not linger when they need to intervene with their present blood glucose, and to go to sleep before midnight to avoid any metabolic complications in the waking hours.It must be noted that, unfortunately, it is impossible in my setting to actually control the metabolisms of the subjects to full extent, not only because this is an observation and not an experiment, which limits my ability to exercise control over the subjects (excluding me) but also because there are some factors that I simply do not have the ability to control, such as the feeling of anxiety or excitement; and also, as the subjects' metabolisms are bound to vary within themselves and among each other, it is also not possible to control the amount of insulin injected in any case (excluding me). To minimize the disparities however, a probation period is set up. During this 24 hour probation period, the subjects are asked to not eat any nutrients with high lipid values or do any sports and try to keep their blood glucose levels between 70-140 mg/dl.

As a note of safety, it must be known that theobservationwas done on willing human subjects that have a case of "Type 1 Diabetes Mellitus". The lack of insulin secretion in the subjects' bodies allow the subjects to be used safely on the observation, as the amount of insulin they get is perfectly under the volunteers respective controls. As glucose level below 70mg/dl is considered a case of hypoglycaemia and is lethally dangerous, and long term exposure to a blood glucose level above 140mg/dl is dangerous to many organs starting with the eyes and the kidney and

[8]Check Appendix-2

[9]Michael Strano, the Charles and Hilda Roddey Associate Professor of Chemical Engineering at MIT. (http://srg.mit.edu/), (http://engineering.mit.edu/ask/how-do-glucometers-work)

[10] Check Appendix-4

officially is considered a hyperglycaemia, it has been requested to these volunteers that they do not exceed these limits and keep their glucose level at tight control, checking their blood glucose levels each 90 minutes and 90 and 135 minutes after each injection during the time of active observation.

METHOD

A copy of the daily program as a time schedule can be found in appendix-3.

1. 24 hour probation period starts according to the specifications in method development.
2. 30 minutes before ending of the mentioned 24 hours, the subjects recheck their blood glucose level to acknowledge any final adjustments to be made with the means of sugar lumps and insulin and treat themselves accordingly to level their blood glucose, aiming for it to stay at a range of 70-140mg/dl, then record the measurements and treatments in their recording book.
3. After the mentioned 24 hours being past, all subjects check their blood glucose level, and record it on their recording book with the value of the blood glucose level in mg/dl and the time of measurement in a 24 hour format with hours and minutes.
4. Each subject takes their predetermined meals[11] in their predetermined amounts and inject themselves with appropriate insulin doses (as these doses cannot be forced by the observer, check method development) according to their bodily settings and start eating their food without hesitation or lingering and try to finish the meal at a maximum of 30 minutes time.
5. Starting from the 90th minute after the insulin injection (beginning of dinner), each subject checks their blood glucose level and records according to the statement at step 2[12].
6. 135 minutes after the insulin injection, each subject checks their blood glucose level again and record it in their recording books. Blood glucose levels above 140 mg/dl and below 70mg/dl are respectively treated with insulin and nutrients by each subjects' appropriate amounts. Treatments alongside with the measurements are recorded in the recording books.
7. Blood glucose level is to be checked every 90 minutes then, each value to be treated accordingly and strictly recorded to each respective subject's notebooks (skip the checking while asleep provided that there are no symptoms), until before the next meal.

[11] Check Appendix-2

[12] After the check, if the blood glucose level of any subject is **below 80mg/dl and is feeling abnormal**, they must **IMMEDIATELY** treat the abnormality by consuming appropriate amount of nutrients. If the blood glucose level of any subject is at or below **70mg/dl**, they should **IMMEDIATELY** treat the abnormality by consuming appropriate amount of nutrients. Nutrient intake in such emergency situations are to be done using supplements with lipid values preferably below 20% as stated before to be able to safely control the amount of glucose in blood in mg/dl. All measurements and treatments done are recorded on the recording book of each respective subject after the initial danger of an hypoglycemic attack has past.

8. Before the next meal, the subjects recheck their blood glucose level to acknowledge any final adjustments to be made by modifying the amount of insulin to be injected before the meal and treat themselves accordingly to level their blood glucose, aiming for it to stay at/reach 140mg/dl and record the measurements and treatments in their recording book. Steps 5 to 10 are repeated for every meal from now for a total of 5 days during the observation, in whichday is made out of 3 main meals and no sub meals but instant intervention by according to blood glucose level.

DATA COLLECTION AND PROCESS

		Subjects	
		Female	Male
Controlled Variables	Species	Human	Human
	Place of residence	Ankara, Turkey	Ankara, Turkey
	Presence of Type 1 Diabetes	Present	Present
	Medical conditions at time being	Healthy	Healthy
	Time of observation	5 days of school	5 days of school
	Age	17	17
	Height (cm)	171.5	173
	Weight (kg)	57	62
	BMI	19.4	20.5

Table 2.1: Raw data of successfully controlled variables of the subjectsalong with their biomass measured via mass, size measured via height and BMI

		Blood Glucose Values (mg/dl ± 20%) in checkpoints between intervals (minutes)													
	Days	Before Breakfast	90	135	225	Before Lunch	90	135	225	315	405	Before Dinner	90	135	225
Female	1	135	112	142		142(same)	156	129	144			144(same)	133	138	
	2	87	85	172	150	104	84	107	109	80		122	168	127	173
	3	129	104	90	178	102	133	158	132	78		124	106	85	160
	4	120	137	82	188	114	123	92	144	139		194	100	89	186
	5	161	92	97	157	96	169	83	164	150		182	112	138	148
Male	1	73	136	122	32(*)	32(same)		117	109			99	141	96(*)	146
	2	41	206	136	55(*)	55(same)	66(*)	116	80(*)	49(*)		77	198	179	218(*)
	3	192	104(*)	79(*)	65(*)	65(same)	76(*)	126	101	82		71	193	226(*)	196
	4	111	128(*)	100	41(*)	41(same)	110	194	323(*)	166	79	59	122	107	85(*)
	5	52	163	154(*)	84	84(same)	80	76(*)	134	133		99	153	110	88

Table 2.2: Raw Data of Measured blood glucose values (mg/dl±20%)in checkpoints between time intervals of the subjects in which the said checkpoints are arranged as 90 minutes after any meal (insulin injection), 45 minutes after that, and then every 90 minutes after that until the next injection throughout the observation where values with an asterisk mean intervention to the blood glucose via insulin or carbohydrate supplements

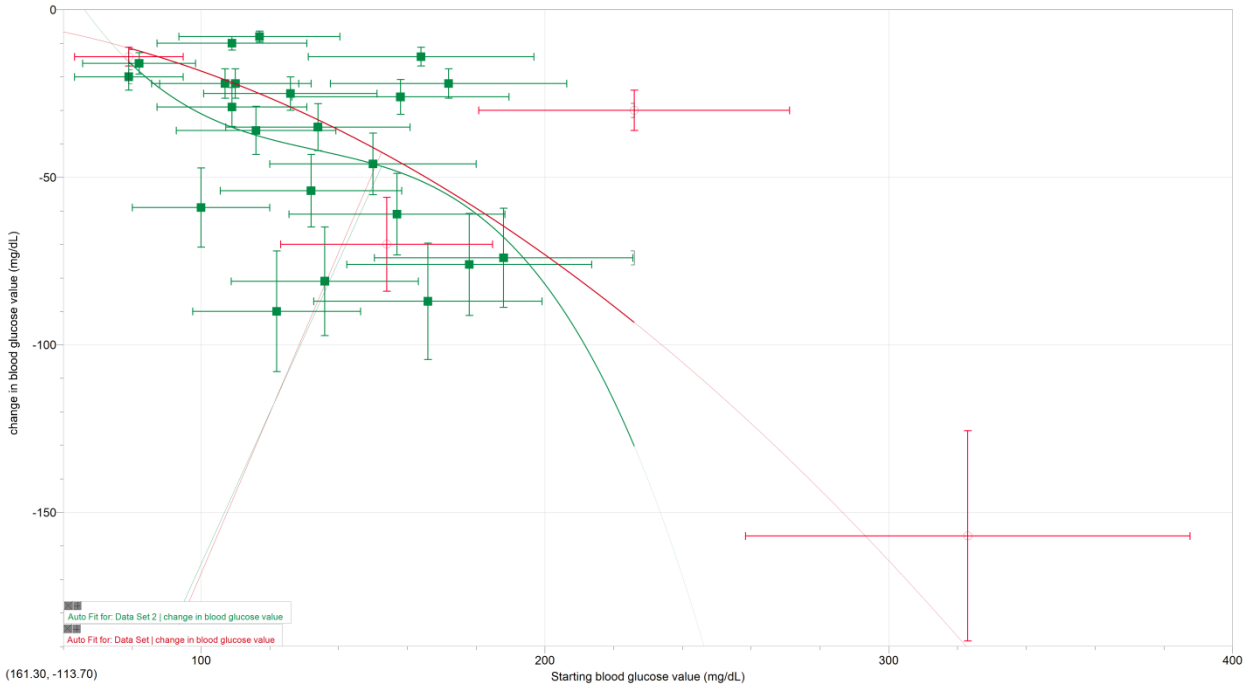
For +ΔG		For -ΔG	
Starting blood glucose value(mg/dl)	ΔG (mg/dl)	Starting blood glucose value(mg/dl)	ΔG (mg/dl)
49*	28	79*	14
76*	58	79	20
78	46	82	16
80	42	100	59
82	106	107	22
83*	81	109	10
85	75	109	29
89	97	110	22
90	88	116	36
92	52	117	8
96	50	122	90
97	60	126	25
127	46	132	54
129	15	134	35
138	10	136	81
139	55	150	46
150	32	154*	70
179	39	157	61
194	129	158	26
		164	14
		166	87
		172	22
		178	76
		188	74
		226*	30
		323*	157

Table 2.3: Initial glucose values measuredstarting from 135 minutes after any meal until any new injection and their change in a 90 minutes period in which thus ΔG values are classified according to whether their trends are negative or positiveandvalues with an asterisk point to an intervention to the blood glucose via insulin or carbohydrate supplements where it should be noted that the first 135 minutes after any injection is not used as an initial blood glucose value for the table because of that rapid insulin is the most effective approximately during the first 1 to 2 hours after injection[13]

A graph for the negative trend is needed at this point as having an idea about the rate of decline is essential for the research. The uncertainties in the graphs are shown as 20% as it the maximum

[13]http://www.webmd.com/diabetes/guide/diabetes-types-insulin

uncertainty let by ISO 15197:2003criteria and according to Abbott Diabetes Care for and Pub Med Central, both of the used Glucometers suit these parameters[14].



.

Graph 2.1: Initial glucose values measured at least 135 minutes after any meal and their negative change in a 90 minutes period in which the red values are the ones that have been acted upon and green ones are the ones that were not intervened with and the last data is ignored due to that there aren't any other values by its uncertainty range.

At this point from the graph, it seems like the rate of decline in average increases with increasing initial blood glucose level even when the values that are the result of interventions are ruled out. To make sure if this difference in rate actually means anything or is circumstantial, a paired samples t test is run on the system.

A paired samples t test is run because there are two independent groups to be considered to check the presence of a threshold: blood glucose (G) values above a point, and below a point. To be able to use the maximum amount of data (as t test requires data sets used in it to be equivalent), the threshold was hypothesized to be 130 mg/dl.With an α of 0.05, the following tables unfold:

[14] http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3045246/ for Contour TS and https://abbottdiabetescare.co.uk/images/uploads/documents/CP075.pdf for Optium Freestyle Lite

		ΔG when; Initial G below 130 mg/dl	Initial G above 130 mg/dl
Trials	1	-20	-54
	2	-16	-35
	3	-59	-81
	4	-22	-46
	5	-10	-61
	6	-29	-26
	7	-22	-14
	8	-36	-87
	9	-8	-22
	10	-90	-76
	11	-25	-74

Table 2.4:ΔG values from initial glucose values measured at least 135 minutes after any meal and their decreasing change in a 90 minutes period in which the values that were intervened with are not used and those that were not intervened with are split asbelow and above 130 mg/dl in order to check for whether or not there is a threshold for blood glucose level in which once below it, its rate of decline increases

	Initial G below 130 mg/dl	*Initial G above 130 mg/dl*
Mean	-30.6364	-52.3636364
Variance	580.6545	655.4545455
Observations	11	11
Pearson Correlation	0.55995	
Hypothesized Mean Difference	0	
df	10	
t Stat	3.086145	
P(T<=t) one-tail	0.00576	
t Critical one-tail	1.812461	
P(T<=t) two-tail	0.011519	
t Critical two-tail	2.228139	

Table 2.5: Paired samples t test of blood glucose levelsat least 135 minutes after any meal and their negative change in a 90 minutes period when the initial blood glucose levels are split as below and above 130 mg/dl in order to check for whether or not there is a threshold for blood glucose level in which once below it, its rate of decline increases

CONCLUSION AND EVALUATION

During the observations and the analysis that followed, whether there was a threshold or notfor blood glucose level in which once above it, its rate of decline would increase was aimed to be found out. It was hypothesized due to my incidental observations in the past and my resulting intuitionand my reasoning suggesting that insulin in blood above a point would result in hypoglycaemia, resulting in the host body eating to recover from thus hypoglycaemia, resulting in more insulin secretion, resulting in fast drops in blood glucose after a certain point, that such a threshold was indeed present.

In graph 2.1, it seemed the rate of decline would increase as the initial blood glucose level increased, compliant to that which was hypothesized. Thus, to check whether this trend was definite enough to be named such a threshold and because there were two independent variables in what was sought out (ΔG above 130 and below 130 mg/dl), a paired samples t test was done with a presumed threshold of 130 mg/dl as only with this presumed threshold all the declining values in table 2.4 could be used (T-test requires the number of data in each data set to be same). It should also be noted that the presumed threshold being at 130 mg/dl is relatively convenient as this value is in the blood glucose safety margins stated before.The resulting P(T<=t) one tail value was 0.00576, which is lower than the alpha value of 0.05, making the found difference between the means of the ΔG values above and below 130 mg/dl meaningful, suggesting that such a threshold in the vicinities of 130 mg/dl of blood glucose is real and compliant with the hypothesis; meaning that **there is indeed a threshold for blood glucose in which once above it, its decline rate will increase,** consequently accepting the hypothesis**.**

A potentially logical explanation depends actually on a simple understanding of gradients: It is known through the rules diffusion that, spontaneously, matter tends to move from where it is more concentrated to where it is less concentrated; if one would apply this piece of knowledge for blood and glucose, they would be able say that as after glucose channels on the cells are opened by insulin, blood glucose passes through not via active transport, but via diffusion; therefore the more the gradient between platforms, the more the intensity of diffusion. Therefore as this gradient decreases with decreasing blood glucose value, so does the blood glucose's rate of decline.

CONCLUSION

As someone having type 1 Diabetes,my main reason in conducting this research was to create some kind of method to simplify keeping track of blood glucose levels especially when in danger of hypoglycaemia: I was aiming to find out whether or not the possibility of hypoglycaemias could be predictedif the trend of the fall can be ascertained. Thus, upon my said incidental observation, I thought that blood glucose may start declining at a faster rate when its above a certain value, hence making it possible to determine whether any current decline in blood glucose is dangerous and should be acted upon or be rechecked in a little while or not via whether said initial blood glucose level is above or below the threshold. Having this piece of information would be extremely useful when one is short of Glucometer lancets and has to use as few of them as possible.

If the outcomes of this investigation are correct, then it is vital to notice declines in blood glucose values especially when they are above the safe blood glucose level margins, bluntly 130 mg/dl as when blood glucose is above this threshold, it becomes more susceptible to declining, especially due to any agitations, namely intervention via insulin and/or physical or mental exertion, leading to cases of hypoglycaemia.

This phenomenon is particularly dangerous for people in certain diets, namely diets that seek to make the body lose weight through long terms of near starvation and eating rarely and for people that frequently consume snacks and sweets without care because when nutrients are consumed after a very long period fasting, or when nutrients of large glucose percentages are consumed much and frequently, they tend to make the blood glucose rise very quickly, stimulating the secretion of insulin along with it, consequently causing hypoglycaemia due to large initial gradient between blood glucose and glucose in cells.

For a general implementation of this phenomenon in industry, Glucometers could be made to warn the user when their blood glucose values go above a certain amount after 130 mg/dl so that the said user can react accordingly.

EVALUATION

Even though it is not possible to assess whether the results were defective or not as the they were consistent among themselves, it can be said that there are flaws in the method. As stated before, the most visible of these flaws is the insufficiency in the number of treatments and trials, namely the volunteers being limited to 2 people due to the limited availability of diabetic 12th graders (Choosing any other students of different ages would increase uncertainties by quite an amount as hormones vary significantly in teen ages) and the limited time frame in which the observations could take place (The period in which there are no exams are practically limited to one week per semester and metabolic activity is abnormal in the course of stressful activities during weeks that exams are, making it impractical to take observations in these periods), resulting in a small

numberof blood glucose trials for a small number of people, consequently increasing the possibility (while not ensuring it) that the results are offshoots.

As human experimentations are prohibited, a diet could not be forced to the specimens. Instead, the investigation was run as an observation, via volunteering specimens with a mutually arranged diet and set blood glucose checking checkpoints, which is, intentionally or not, violated on instances (using different nutrients than those that are agreed on in instances they cannot be found or being unable to check blood glucose due to being unable to reach the Glucometer temporarily for example), causing large uncertainties in the results. There are two possible solutions to this limitation, both of which are virtually impossible for me to do. The first of these solutions would be giving the principles of this observation to doctors and pharmacists, so that they could via volunteering subjects experiment on the subjects instead of observing them, exercising full control over them; while the second solution would be giving the principles of this observation to scientists for animal experimentation, so that even though the accuracy of the experiment for the human kind would potentially decrease, its precision would increase because of increasing sample amount and available time frame, potentially leading to clearer judgments in at least the species that were experimented on.

Extensive observations can be conducted wholly on more than one male or female specimen or the same setting this investigation is on can be redone with a larger number of volunteers would there be any to have a more specific understanding on the working principles of this threshold now that its presence is known. Also, changes in the incoming and circulating cell traffic of chemicals and nutrients can be investigated above and below this threshold to actually see the rate of these when it becomes possible to see the insides of a cell in sufficiently high resolution. This will probably be possible shortly with the most recent advances in microscopes, namely the studies regarding microsphere nanoscopes[15], and will also allow the mechanism behind this phenomenon to be literally seen.

It should be noted that the setting of this observation could also be repeated for other relationships based on gradients or gradient-like mechanisms, such as the effect of deposited or blood Ca^{2+} on the amount of secreted parathormone and calcitonin, in which the subjects have either calcitonin or parathormone deficiency so that either one of those factors can be independently controlled while their change to deposited or blood Ca^{2+} is observed.

[15] http://www.wired.com/2011/03/microsphere-nanoscope/

APPENDIX-1

RESEARCH MEDICAL DOCTOR CONSENT FORM

I, medical doctor Sibel Tüzün Klnik, by signing this form, recognize that the investigation conducted by the International Baccalaureate student Kaan ÜNLÜ as part of the compulsory Extended Essay based on observation of a potential threshold in blood glucose level in which once below it, the amount of blood glucose is theorized to be declining more rapidly shall be conducted within the everyday, or otherwise known as safe margins (72-140mg/dL of blood glucose) of the subjects and does not in whatsoever any way force them to take extreme measures at any part, or take part in the observation at all and is therefore completely safe regarding the subjects daily health and overall survivability if not formidably abused/sabotaged by any source.

Date, Name, Signature

4th, Apr 2015

Prof. Dr Sibel Tüzün Kuh

APPENDIX-2

DETERMINED DIET

BREAKFAST

- A toast prepared from 2 25 grams of white bread of the brand UNO and cheese between it,
- 200ml semi-skimmed milk;

LUNCH

- 2 sandwiches prepared from 72 grams of sandwich bread of the brand UNO with contents that are chiefly lipid-free and worth less than 1 dose of rapid insulin;

DINNER

- 400 grams of sauce-free pasta

Any other intake of nutrients besides immediate reactions to glucose level is strictly prohibited as such will interfere with the accuracy of the values measured from during the checkpoints. Apart from that, consumption of snacks such as cucumbers and green leaves are allowed due to their negligible lipid and digestible carbohydrate composition.

APPENDIX-3

DAILY SCHEDULE

The following schedule model assumes that the breakfast is eaten at 6.35 and the observation proceeds in both home and school in which the each blood glucose checkpoint in school is slightly modified to suit the breaks between each class.

06:35	08:10	08:55	09:45	10:40	11:30	13:05	13:55	14:50
Breakfast	90 minute check	135 minute check		Routine check	Lunch	90 minute check	135 minute check	
Home	School							
15:40	17:10	18:40	19:10	20:40	21:25	22:55	00:25	
Routine check	Routine check	Routine check	Dinner	90 minute check	135 minute check	Routine check	Routine check	
School	Home							

APPENDIX-4

EQUIPMENT

- The male used;
  - Freestyle Optium Lite Glucometer
  - Novo Rapid Insulin Aspart
  - Levemir Insulin Detemir
- The female used;
  - Contour TS Glucometer
  - Humalog Insulin Lispro
  - Levemir Insulin Detemir

BIBLIOGRAPHY

WEBSITES

- "Type 1 Diabetes - Causes ." *Type 1 Diabetes*. NHS Choices, 2014. Web. 21 Jan. 2016. <http://www.nhs.uk/Conditions/Diabetes-type1/Pages/Causes.aspx>.

- "Hypoglycemia (Diabetic) & Hyperglycemia." *@healthcentral*. Remedy Health Media, LLC, n.d. Web. 21 Jan. 2016. <http://www.healthcentral.com/encyclopedia/hc/hypoglycemia---hyperglycemia-3168893/>.

- "The weight of nations: an estimation of adult human biomass", Sarah Catherine Walpole, David Prieto-Merino, Phil Edwards, John Cleland, Gretchen Stevens and Ian Roberts, BMC Public health article

- Bowen, R. "Insulin Synthesis and Secretion." *Insulin Synthesis and Secretion*. N.p., 15 June 1999. Web. 21 Jan. 2016. <http://www.vivo.colostate.edu/hbooks/pathphys/endocrine/pancreas/insulin.html>.

- Strano, Michael, the Charles and Hilda Roddey Associate Professor of Chemical Engineering at MIT. "How Do Glucometers Work?" *Ask an Engineer*. N.p., 18 Oct. 2011. Web. 21 Jan. 2016. <http://engineering.mit.edu/ask/how-do-glucometers-work>.

- "Brands and Types of Insulin: Rapid-Acting, Long-Acting, and More." *WebMD*. Ed. Michael Dansinger. WebMD, 4 Dec. 2015. Web. 21 Jan. 2016. <http://www.webmd.com/diabetes/guide/diabetes-types-insulin>.

- Frank, Joy, Jane F. Wallace, Scott Pardo, and Joan Lee Parkes. "Performance of the CONTOUR® TS Blood Glucose Monitoring System." PubMed Central (PMC), 1 Jan. 2011. Web. 21 Jan. 2016. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3045246/>.

- Abbott Laboratories, Inc., comp. "Accuracy Evaluation of the FreeStyle Freedom Lite, Accu-Chek Performa, OneTouch Ultra and OneTouch Vita Systems." *Accuracy of Four Blood Glucose Monitoring Systems* (n.d.): n. pag. Web. <https://abbottdiabetescare.co.uk/images/uploads/documents/CP075.pdf>.

- Mosher, Dave. "Tiny Spheres Turn Regular Microscopes Into Nanoscopes." Wired, 3 Jan. 2011. Web. 21 Jan. 2016. <http://www.wired.com/2011/03/microsphere-nanoscope/>.