석 사 학 위 논 문

OPTIMIZATION OF GLUCOSE AND LACTATE ASSAY IN BLOOD PLASMA FOR HEALTH MONITORING

계 명 대 학 교 대 학 원

의 학 과

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지 도 교 수 허 윤 석

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이 논문을 석사학위 논문으로 제출함

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T H A P A S E E M A

타파 시마의 석사학위 논문을 인준함

주 심 박 희 준

부 심 허 윤 석

부 심 김 선 칠

계 명 대 학 교 대 학 원

2 0 1 9 년 8 월

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2019 년 8 월

THAPA SEEMA

Abbreviation

Full form	Abbreviation
adenosine triphosphate	ATP
nicotinamide adenine dinucleotide	NADH
millimole/liter	mmol/L
milligram/deciliter	mg/dl
millimolar	mM
hydrogen peroxide	H2O2
horseradish peroxidase	HRP
units/milliliter	U/ml
nanometer(s)	nM
glucose Oxidase	GOX
lactate Oxidase	LOX
micromole	μM
microliter	μl
gram	gm
high fat diet	HFD
lactate/glucose	L/G
standard deviation	SD

Table of Contents

1. Introduction ·····1
1.1 Assay Principle ·····5
2. Materials and Methods ·····10
2.1 Animal Model and Diets ·····10
2.2 Blood Sampling ·····10
2.3 Assay Materials ·····11
2.3 Assay Plates ·····12
2.4 Fluorescence Assay ·····12
2.5 Statistical Analysis ·····13
3. Results ·····17
3.1 Mice weight Record ·····17
3.2 Assay Optimization ·····17
3.3 Assay Results ·····18
3.4 Statistical analysis and its Result ·····18
4. Discussion ·····26
5. Summary ·····28
References ·····29
Abstract ·····38
국문초록 ·····40

List of Tables

Table 1. Properties of Different Types of Plates used in Assays ····14

Table 2. Comparison Chart of Glucose level in C57Bl/6j Strain Mice (Reference Papers) ····15

Table 3. Comparison Chart of Lactate Assay (Reference Papers) ····16

List of Figures

Figure 1. Schematic diagram of glucose and lactate assay. ……8

Figure 2. Schematic diagram of overall assay procedure. ……10

Figure 3. Mice body weight gain from 7-16 weeks of their age. ……19

Figure 4. Average body weight gain of normal and HFD fed mice ……20

Figure 5. Glucose and lactate assay standards plotted time vs. fluorescence ……21

Figure 6. Glucose assay standard plotted concentration vs. fluorescence ……22

Figure 7. Lactate assay standard plotted concentration vs. fluorescence …… 23

Figure 8. Glucose and lactate level of mice plotted against their body weight. ……24

Figure 9. Blood plasma glucose level plotted against L/G ratio……25

1. Introduction

Lactate and glucose both are metabolically significant organic bio-compounds, where glucose and lactate contribute directly or indirectly for the high energy products like ATP and NADH. Glucose is a monosaccharide hexose sugar and the major source of energy which vary in its concentration with respect to food intake in blood. Organisms maintain their blood glucose level as a part of their metabolic homeostasis [1]. Actions of various hormones like insulin and glucagon helps to maintain this homeostasis to prevent diabetes and many other metabolic diseases. In today's world, hyperglycemia and diabetes alone is one of the important cause for mortality rate in the world in association with cardiovascular and kidney diseases [2-4]. Measurement of glucose is very important to determine the diseases like diabetes in time and also for drug discovery. A person weighing 70kg has the 4 gm of blood glucose circulating [1] in the body. Normal blood glucose level in non-diabetic individual before meal ranges from 4 to 5.9 mmol/L (72 to 99 mg/dl) which reaches 7.8 mmol/L (140 mg/dl) to the maximum, 90 minutes after meal. Fasting blood glucose in non-diabetics should range in between 3.9 to 7.1 mmol/L (70 to 130 mg/dl)[5]. Whereas, prediabetes or impaired glucose tolerance should be 5.5 to 6.9 mmol/l (100-125 mg/dl) and person is considered diabetic when his fasting blood plasma glucose level is 7.0 mmol/L (126 mg/dl) or above [6, 7]. However, in case of mice, the blood glucose varies from strain to strain and the hour and timing of the fasting. Normal glucose concentration in 9 hour fasted C57Bl/6J male mice ranges to 5.2± 0.3 [8, 9] whereas ~ 5.5 mM of blood glucose was measured in 6 hour fasted mice [10]. Other glucose

concentrations are shown in Table 2. Fasting mice also show gradual weight loss with the length of fasting hours. More the time of fasting higher will be the body weight loss [11]. Fasted mice is considered as the standard protocol as a model for human diseases and disorders [12]. Fasted blood glucose test is usually done for the diagnosis of diabetes mellitus [13] and also to test the effectiveness of different medication or dietary changes on people diagnosed as diabetic. Among the different mice strains, C57BL/6J mice strain show good effect on dietary obesity [14-17]. High fat diet induced C57BL/6J mice group shows the increased level of glucose [18] than compared to the normal chow fed mice. Studies have found that the fasting mice has the decreased plasma blood glucose with increasing fasting time [8, 19-22]. Plasma glucose also varies with the gender of the mice. Female mice usually has low plasma glucose than compared to male [20]. Apart from this, obese C57BL/6J male mice also show the sign of diabetes [23]. Fasting blood glucose level over 200 mg/dl (11.1 mmol/L) in mice is considered to be diabetic [24]. Random blood sugar level of 200 mg/dl or 11.1 mmol/L or higher is also considered diabetic in human health monitoring. However, for the measurement of blood glucose level, venous plasma glucose is the standard sample. Plasma should be immediately separated from the blood and placed in ice water or should be measured immediately after collection [7]. The storage of blood plasma in room temperature even for few hours decreases the concentration of glucose [25].

Lactate was first discovered in sour milk which was later found in blood of living patient [26]. L- Lactate found in human body is the end product of the glycolysis [27]. Glucose is converted to pyruvate with the help of pyruvate dehydrogenase in the process of glycolysis, where in presence of oxygen, the product pyruvate is changed to

acetyl CoA whereas lactate is formed in anaerobic condition [28]. It is an important intermediary in metabolic processes [29] and plays an important role in many biological processes along with capacity in wound healing. Lactic acid, lactate and sodium lactate all are found naturally in human body, produced by most tissues and especially found in the muscles [30]. Lactic acid is acidic in nature whereas the sodium lactate is basic but the L- lactate in the body is almost in the neutral form. Under physiological PH, Lactic acid produced in the body changes to lactate [29, 31]. Human blood PH when decreases or increases, it might cause in some health problems. The condition where the acid is more in the body (≤7.35) is called acidosis and the condition where base is in excess amount (≥7.45) is called alkalosis (https://www.healthline.com/health/acidosis). This may be due to some problem or improper function of lungs and kidneys. Human blood has two types of lactate; D-lactate and L-lactate being L-lactate higher in concentration. D-lactate is only found in 1-5% of the total lactate concentration [32] and is of the microbial origin.

Under the normal and resting condition the blood lactate concentration in human blood is 0.6 to 2.0 mmol but increases up to 20-30 mmol during the physical activities like exercise [33]. Lactate is produced by cells when the body burns carbohydrate into energy. The lactate is produced in excess amount by muscle cells, red blood cells, brain and other tissues when the oxygen is insufficient. Lactate level is naturally increased during exercise and heavy workout. But the abnormally increased blood lactate level is said to be related with the presence of tissue hypoxia [34], different diseases like diabetes, lactic acidosis and so on. Lactate, so far is the neglected factor for diabetes and even for the cancer interaction.

Lactate level is measured in whole blood, plasma or serum. Normal blood lactate level of human being ranges from 1-1.5 mmol/L [35-37] and plasma lactate concentration ranges from 0.3-13 mmol [38]. Studies have found no any difference in values measured in plasma or serum but lactate level measured in plasma show slightly higher value than measured in whole blood [39]. Whole blood lactate and glucose measurement should be performed as soon as possible. Whole blood lactate measurement should be done within 15 minutes whereas, for glucose it should be finished within 30 minutes to prevent the possible in-vitro metabolism [40]. Samples whether whole blood, plasma or serum is not recommended to store for long time even if they are frozen at -20°C due to the chances of decreasing in glucose level. However, storage in -4°C showed less loss of glucose than stored in -20°C [41]. Plasma samples are recommended to measure blood glucose if little delay is expected since it is more stable compared to other [42].

In mice, lactate level is quite variable from stain to strain. $BLAB_c$ mice has blood lactate concentrations 4.6±0.7 mmol at 7.39±0.026 blood PH [43]. However, studies have found that the fasting mice has the decreased blood glucose [8, 19, 20] and lactate level [19]. In recent studies, obese diabetic patients are found to have higher amount of fasting plasma lactate level than obese nondiabetic individuals [44, 45]. It is also found that the lactate is related to the type 2 diabetes [46].

Lactate in critically ill patients acts as an indicator of the possible outcome [47, 48]. The rate of mortality becomes higher with the elevation of lactate level in body [49]. Apart from this, the lactate is found to be associated with type two diabetes [38, 50]. Also, increased blood lactate level is said to be related with the

presence of tissue hypoxia [34], lactic acidosis etc. Like glucose, lactate is also elevated among obese subjects and seem to decrease with decreasing obesity [51, 52]. Lactate and glucose both also acts as an indicator in critically ill patients because hyperglycemia is associated with mortality rate in critically ill patients in hospital especially in patients with undiagnosed diabetes [53]. Rapid monitoring of glucose and lactate is necessary for managing critically ill patients suffering from acute myocardial infarction, seizures, shocks and also diabetic patients. Even though the glucose and lactate are associated with each other as an important marker in critically ill patients [40, 47], they are still studied separately. In our study, we have tried to study both indicator; glucose and lactate using their corresponding enzyme same substrate, Amplex red using the single kit material simultaneously.

1.1 Assay Principle

Amplex red is a colorless and non-fluorescent compound that is used as a probe for the measurement of extracellular H2O2, but because H2O2 is freely diffusible, this measurement is an indication of cellular H2O2 production. Amplex red reacts with H2O2 at 1:1 stoichiometric ratio catalyzed by horseradish peroxidase (HRP) to form the colored and highly fluorescent compound resorufin [54]. amplex red can also be used to measure peroxidase activity and can detect as little as 1 × 10^-5 U/mL HRP or, for H2O2, as little as 50 nM [54]. However, at high H2O2 concentrations the concentration of amplex red becomes limiting and resorufin can be further oxidized by HRP to non-fluorescent, colorless product resazurin [55, 56].

Resorufin can also be reduced to form hydroresorufin which is both colorless and non-fluorescent. Resorufin is also formed artificially when amplex red is exposed to the light even in the absence of HRP and H2O2 [57]. HRP catalyzes the oxidation of amplex red via two one-electron oxidation steps in which an amplex red radical intermediate is formed [58]. HRP found in the plant are used extensively in molecular biology and biochemistry primarily for its ability to amplify a weak signal and increase detectability of a target molecule [59].

1.1.1 Glucose Assay

In glucose assay, the enzyme glucose oxidase reacts with D-glucose to form D- gluconolactone and H2O2[60]. H2O2 so produced reacts with amplex red in the presence of HRP (1:1 stoichiometry) to give pink/red fluorescent oxidation product known as resorufin [54, 61].

Glucose oxidase + D- glucose ---------- D - Gluconolactone + H_2O_2

Amplex red + H_2O_2 -------HRP--------- Resorufin

1.1.2 Lactate Assay

Like in glucose assay, pyruvate is produced along with H2O2 when lactate reacts with lactate oxidase. Colorless and non-fluorescent amplex red when reacts with H2O2 so produced gives the fluorescent product, resorufin. Resorufin has the fluorescence excitation maxima of approximately 571 nm and emission maxima of 585 nm. Both the assays are carried out fluorometrically.

Lactate + O2 -----LOX----- Pyruvate + H2O2 [62]

Amplex red + H2O2 -----HRP---- Resorufin

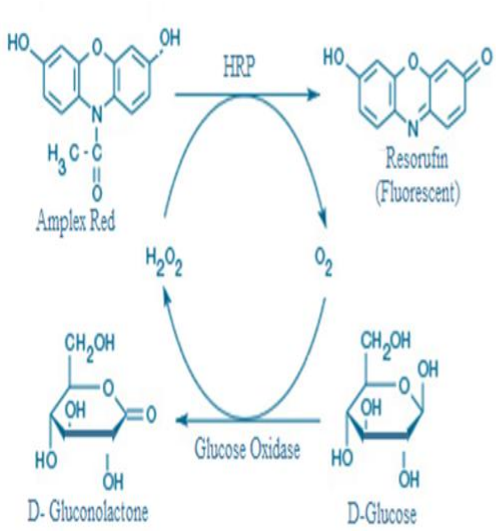

Figure 1: Schematic diagram of Glucose Assay reaction procedure.

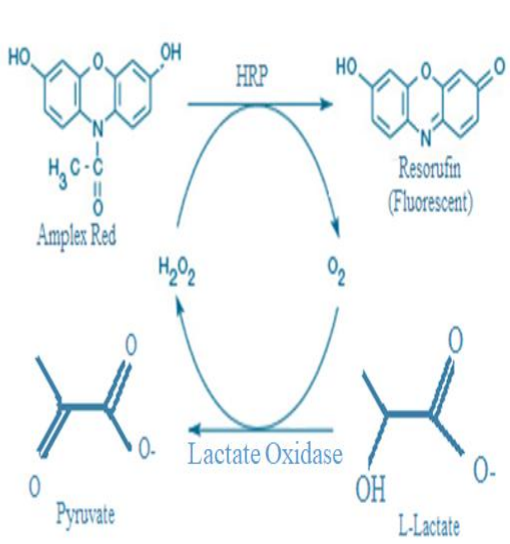

Figure 2: Schematic diagram of Lactate Assay reaction procedure.

Figure 1. Schematic diagram of Glucose and Lactate Assay.

Glucose oxidase enzyme used in glucose assay is a flavo-protein consisting of 2 moles of flavin adenine dinucleotide (FAD) per mole enzyme and catalyzes the oxidation of β-D glucose by molecular oxygen to D-glucose-1, 5-lactone and hydrogen peroxide [63]. It has been isolated from various sources, however, only the enzyme from *Aspergillus niger* and *Penicillium amagasakiense* has been studied in detail [64]. Glucose oxidase is used in the food industry for the removal of glucose from powdered eggs, for gluconic acid production, and as a source of H2O2 in food preservation [65]. Glucose oxidase is used extensively for the quantitative determination of D-glucose in samples such as blood, food and fermentation products [66].
H2O2, on the other hand, generated as a by-product in the above equation is a good oxidizing agent and in the presence of a reducing agent such as o-dianisidine, is catalytically reduced to water by the enzyme HRP.

H2O2 + o-dianisidine ------HRP----- oxidized o-dianisidine + H2O (2)

The overall assay principle is shown in the figure 2. Vector diagram of mice used in the figure is adapted and modified from Wikimedia commons [67].

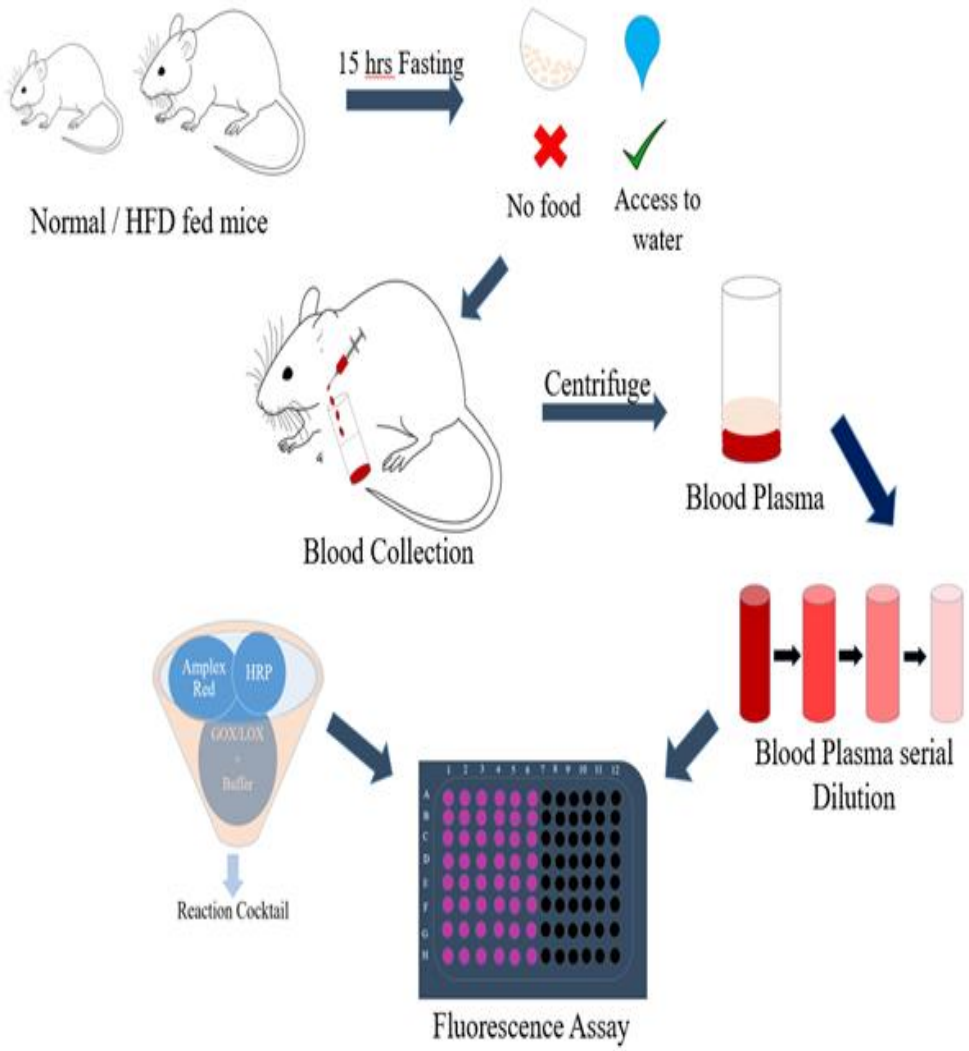

Figure 2. Schematic diagram of overall assay procedure.

2. Materials and Methods

2.1. Animal models and diets

Six to eight weeks male C57BL/6J inbred mice were housed in a controlled environment with a 12-h light/dark cycle (21-23° C) with ad libitum access of water. Two group of mice; in which one group were fed with normal chow and the other group with high fat diet chow were ordered form 하나 상사(South Korea). All experiments were performed in 15 h overnight fasted mice of age group ranging from 6-8 weeks of normal chow fed mice and over 1-month high fat diet fed mice. Fasted mice were deprived of food but not with water. All the experiments performed were approved by the ethics committee for Animal Experiments of the School of Medicine, Keimyung University.

2.2. Blood sampling

Blood was obtained from submandibular vein of overnight fasted mice in heparinized tube as heparin does not affect the measurement of glucose and lactate assay [40]. Blood collection in this method does not need anesthesia so it avoids the effect of anesthetics in blood results and other data. Also, the blood flow can be stopped easily when the required amount is collected and the mice remains unaffected and starts to move around in few minutes. Samples collected were centrifuged directly to separate plasma. Both glucose and lactate was measured as soon as possible after the blood collection and plasma separation.

2.3. Assay Materials

Invitrogen, the amplex red glucose/ glucose oxidase assay kit used in the experiment was ordered from Thermo Fisher Scientific which included reaction catalyst HRP, reaction probe amplex red, glucose and glucose oxidase. Likewise, lactic acid solution and lactate oxidase for lactate assay was ordered from Sigma- Aldrich and My BioSource respectively. Whereas, the Synergy 2 fluorescence reader from the Biotek was used for fluorescence reading.

2.3.1. Glucose Assay

Glucose assay was carried out using commercially purchased assay kit which included reaction catalyst HRP, reaction probe; amplex red, buffer, glucose and glucose oxidase.

Different concentrations of glucose were prepared by using phosphate buffer provided. Likewise, the reaction cocktail was prepared by following the protocol of the kit. Reaction was carried out by mixing 50 µl of reaction cocktail in 50 µl of different concentrations of glucose. The reading was taken using the fluorescence Synergy 2 multi-mode micro-plate reader from BioTek in every 5 minutes for 30 minutes. Standard curve was plotted from the data obtained every 5 minutes up to 30 minutes. The slope intercept was calculated with the help of excel for 30 minutes readings and was used to calculate the amount of glucose present in the blood plasma sample.

2.3.2. Lactate Assay

Lactate assay was also carried out using the kit materials except for the sample lactic acid (Sigma Aldrich) and enzyme lactate oxidase

(My BioSource). Different concentrations of lactic acid as in glucose assay were prepared by using the phosphate buffer. The reaction cocktail used to carry out the reaction was prepared like in the assay for glucose. Likewise, the reactions were carried out by mixing 50 µl of reaction cocktail with 50 µl of different concentrations of lactic acid. However, readings were taken every 5 minutes unlike in glucose assay. Standard curve and slope intercept was plotted as in the same way as glucose to determine the blood plasma lactate level.

2.4. Assay Plates

The critical factor that determines the proper plate type is reflective property. The main difference between a black and a white plate is the reflective property. The plate used for luminescent or fluorescent assays comes with most important feature of opaque walls to prevent well-to-well cross talk. Opaque white plates are used for luminescence and for multiplexing assays that involve both luminescence and fluorescence. Whereas, black plates are used for fluorescence assays. However, for absorbance assays, a clear-bottom plate is needed. For applications in which UV wavelength measurement is needed, such as nucleic acid quantitation, UV-transparent multi well plates should be used.

2.5 Fluorescence Assay

Fluorescence was measured with Synergy 2 microplate reader (BioTek Instruments, Vermont, USA) using 96-well black assay microplate. Glucose and lactate standards were measured with 50 µl volumes of

(5,10,20,30,40,50,60,70,80,90 and 100) μM/well and standard curves were plotted. Plasma glucose and lactate were measured by diluting the sample to 100 times and the concentrations were evaluated with the help of standard curve plotted already. Reading was taken every 5 minute up to 30 minutes for glucose assay and only for 5 minutes in lactate assay. Fluorescence was measured with the excitation of 540/35 and emission of 600/40. Background was corrected by subtracting the value of no-glucose and no-lactate control from all the sample readings.

2.6 Statistical Analysis

Data are expressed as mean± SD data and graphs were made and analysed using the Origin pro 2019.

Table 1. Properties of Different Types of Plates used in Assays.

Properties	Black Plates	White Plates	Clear Plates
Reflective Properties	Reflect less light than white plates Have much lower luminescence than the same assay measured in a white plate.	Reflect more light when measuring a fluorescent assay Cause an increase in assay signal and background.	For absorbance assays. UV-transparent plates used when measurement involves a wavelength in the UV spectrum.
Luminescent Signal	Lower than white plates or clear plates.	Higher than black plates.	Lower than a white plate.
Background	Lower than white plates or clear plates.	Higher than black plates.	
Cross Talk	Lower than white plates or clear plates.	Lower than white plates and clear plates.	Higher than in opaque plates; can be minimized with a top-reading instrument.

Table 2. Comparison Chart of Glucose Level in C57Bl/6j Strain Mice.

S.N.	Refe-rences	Mouse Strain	Fasting Time	Normal Chow Diet		High Fat Diet chow	
				M	F	M	F
1.	[8]	C57BL/6J	Non-fasted	8.8±0.3 mmol/L			
2.	[20]	C57BL/6J	14-16 hrs. Overnight	6.0 ± 0.14 mmol/L	5.6±0.18 mmol/l	6.8± 0.14 mmol/L	7.2± 0.22 mmol/L
3.	[10]	C57BL/6J (Plasma)	6 hrs.	5.6 mM			
4.	[20]	C57BL/6J	12 hrs.	7.0±0.27 mmol/L	7.7±0.25 mmol/L		
5.	[68]	White stock mice (Plasma)	Non-fasted	219 mg/dl			
6.	[24]	C57BL/6J (8Weeks)	12 hrs. Overnight	85.11±22 mg/dl		126.3±26.4mg/dl	
7.	[18]	C57BL/6J (4Weeks)	Non-fasted		7.0±0.1 mmol/L		
8.	[15]	C57BL/6J (8Weeks)	Non-fasted	6.73 ± 0.45 mmol/L		6.91±0.70 mmol/L	
			6 hrs.	7.88±0.42 mmol/L		9.10± 0.31 mmol/L	
			18hrs.	6.13±0.22 mmol/L		6.28±0.51 mmol/L	
9.	[69]	C57BL/6J	5 hrs. (day)	260.61 mg/dl			

Table 3. Comparison Chart of Lactate Assay (Reference Papers).

S.N.	Refe-rences	Mouse Strain	Age/ Weight	Fasting Time	Normal Chow Diet		High Fat Diet chow	
					M	F	M	F
1.	[43]	BLABc	12 week/ 24.9±1.0g	Non-fasted	4.6±0.7mM			
2.	[68]	White stock mice (plasma	NA	NA	59±10(mg/dl)			
3.	[70]	C57BL/6 (Whole blood)	8 W		1.76±0.16 mmol/L		1.44±0.20 mmol/L	
4.	[71]	Hsd:ICR (resting)	NA	NA	2.0-4.8 mmol/L			
		Wheel running			2.8-11.5 mmol/L			

3. Results

3.1 Mice Weight Record

Six weeks age mice were introduced to high fat diet and normal chow diet with initial body weight of 19.18±0.6 gm. Change in body weight were recorded for 27 weeks of their age. Mice fed with high fat diet gains more weight significantly than chow fed mice [15]. Only after one week of high fat diet introduction, mice weight significantly increased by 3.1± 0.6 gm. Whereas, in normal chow fed mice group the weight gain was increased by 1.6±0.8 gm showing higher weight gain in HFD fed mice. Even though the similar pattern in two groups of mice were seen up to 12 weeks of their age, the weight started to fluctuate after and showed slow gain of body weight specially in normal chow fed mice. The mice weight record is shown in the figure 3 and 4.

3.2 Assay Optimization

Lactate assay was optimized in this study. The kinetics of the enzymatic reaction was examined by time trace measurements as shown in figure 5. Fluorescence of the different concentrations of lactic acid increased only up to 5 minutes of incubation. Also, the standard curve plotted concentration versus fluorescence gave the linear line proving the accuracy of the measurement procedure. Fasted mice lactate levels were measured using the similar method.

3.3 Assay Results

Glucose level in most of the HFD fed fasted mice (n=9) were higher than normal chow fed mice (n=9). Plasma glucose in HFD fed mice was seen to be increased by 2.66 than in normal chow fed mice. whereas, slight increase was seen in lactate level. Glucose and lactate level plotted against the mice body weight is shown in figure 8.

3.4 Statistical Analysis and its Result

Incubation time for glucose assay was set as 30 minute in room temperature. In this case, the average change in fluorescence from 5 minute to 30 minute was found to be increased by 2650.364. Whereas, in lactate assay the average increase in fluorescence was found to be just 58.63. Ignoring the minute change in fluorescence which have no significance in result we selected 5 minutes as incubation period for lactate assay.

When L/G ratio was plotted against glucose it showed a distinctive transitional plot between normal and HFD fed specimen. This transitional period falls exactly between 2.155-3.17 (L/G) in our study. So, our study recommends that value of L/G <2.155 indicated that the mice might pose a health threat in coming future if this ratio continues to decrease. Whereas, the value >3.17 signify healthy mice group.

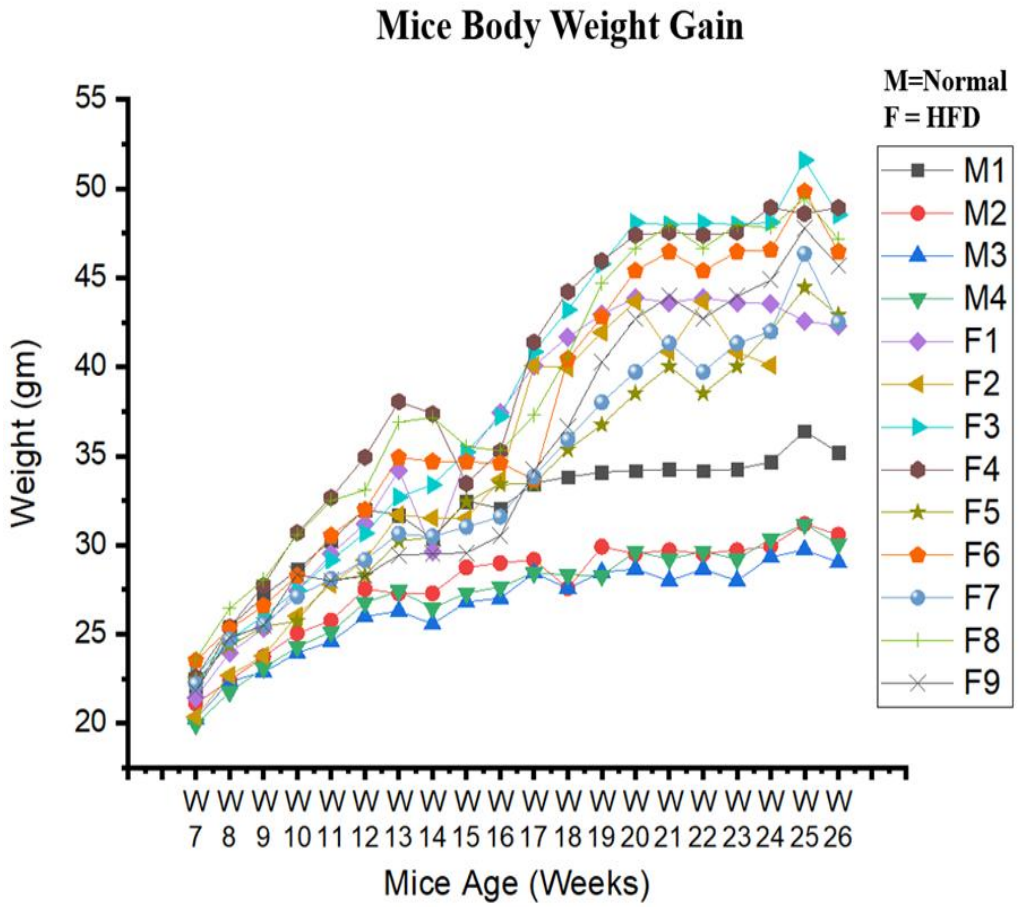

Figure 3. Mice body weight gain from 7 weeks to 27 weeks of their age.

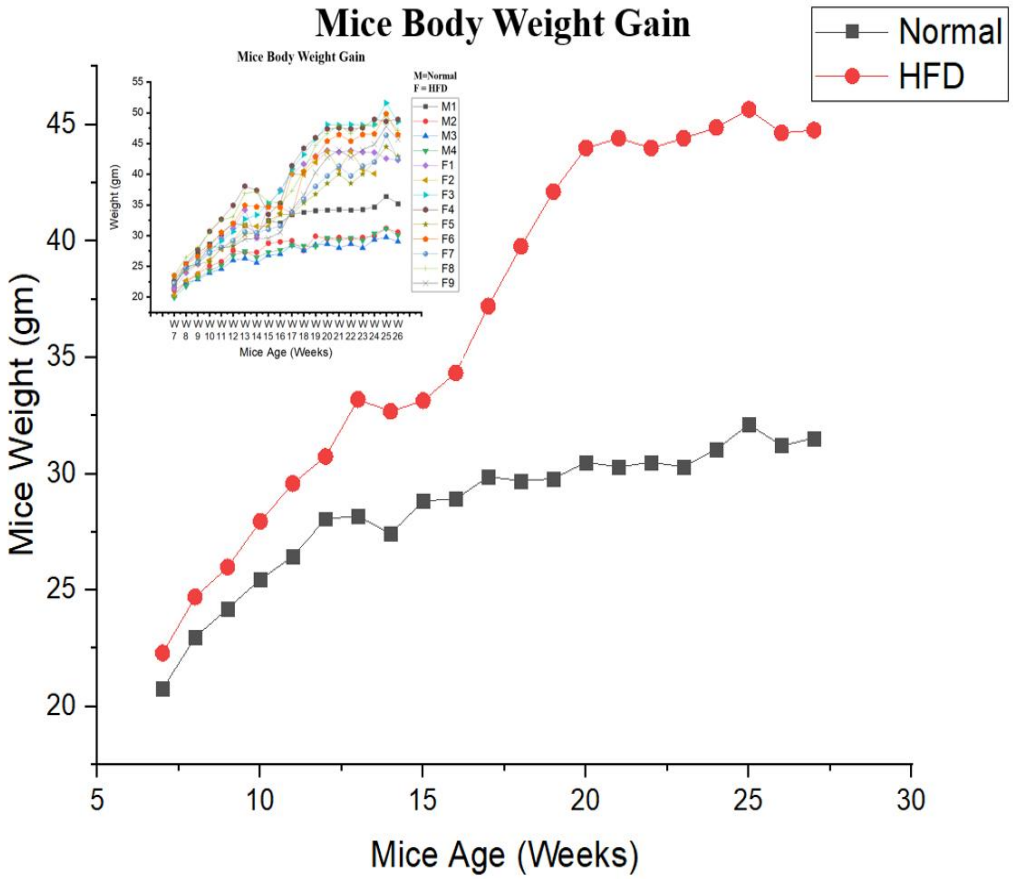

Figure 4. Average weight of normal chow fed and high fat diet fed mice recorded from 7 weeks to 27 weeks of their age.

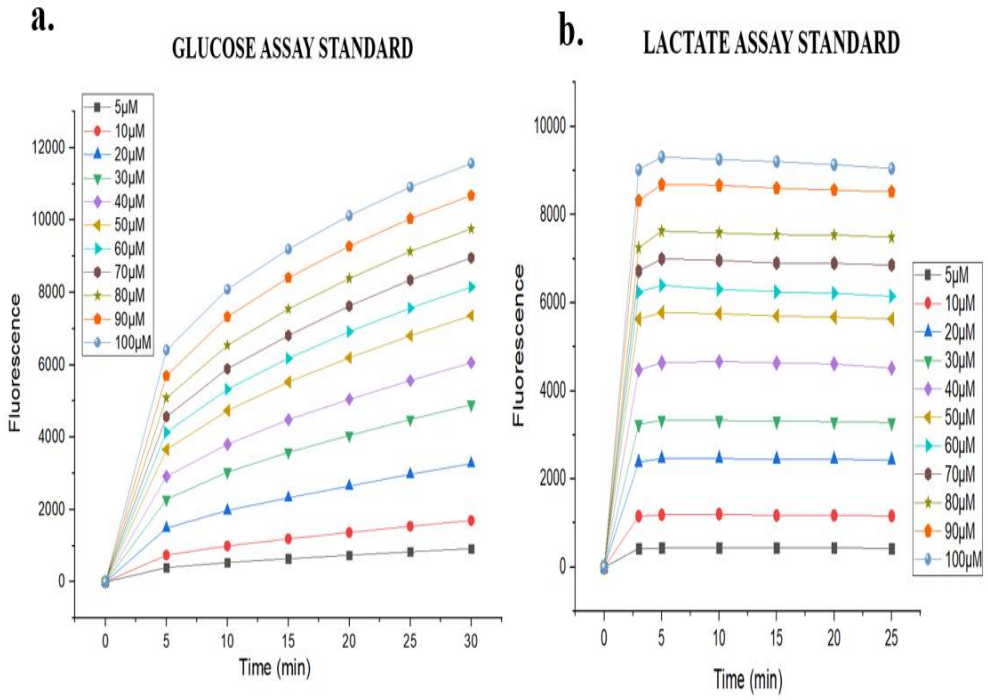

Figure 5. Glucose and lactate assay standards plotted time vs. fluorescence.

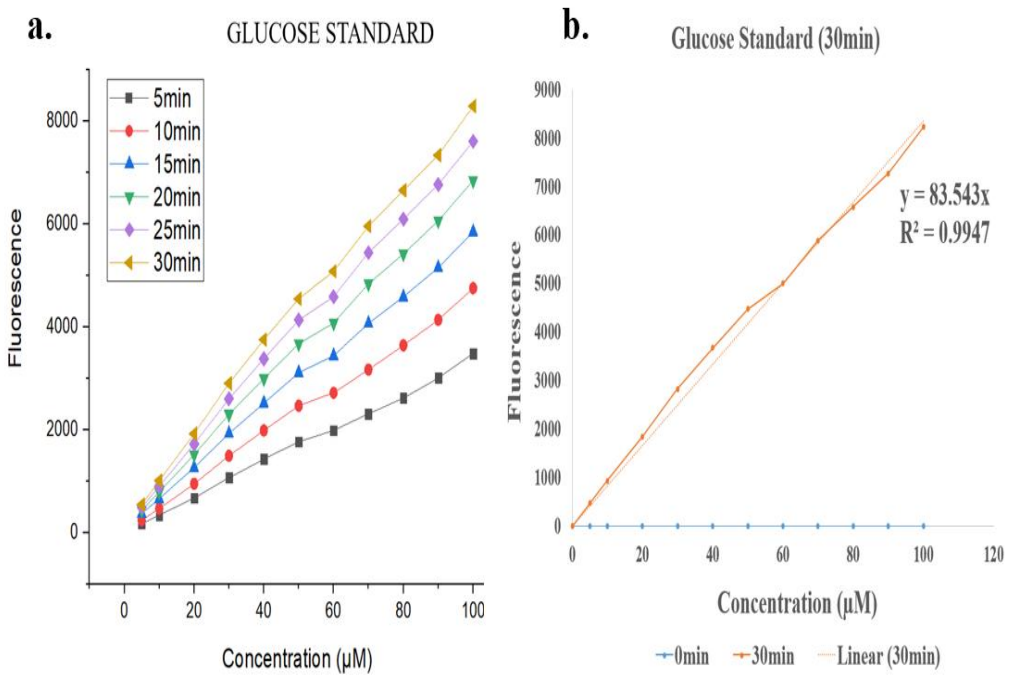

Figure 6. Glucose assay standard plot. (a) showing the fluorescence intensity of different concentrations of glucose in 5 interval of time period up to 30 minutes. (b) The slope intercept form of glucose fluorescence measured at 30 minutes to find unknown concentrations of blood glucose.

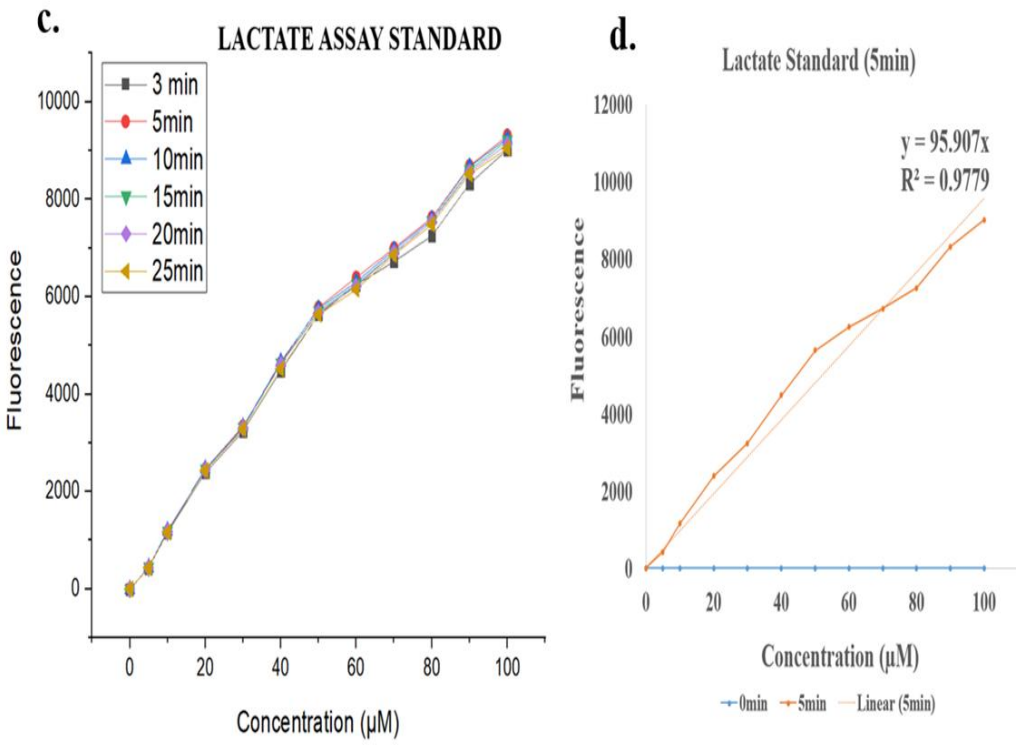

Figure 7. Lactate assay standard plot. (c) showing the fluorescence intensity of different concentrations of lactic acid. (d) The slope intercept form of lactic acid fluorescence measured at 5 minutes to find unknown concentrations of blood plasma lactate.

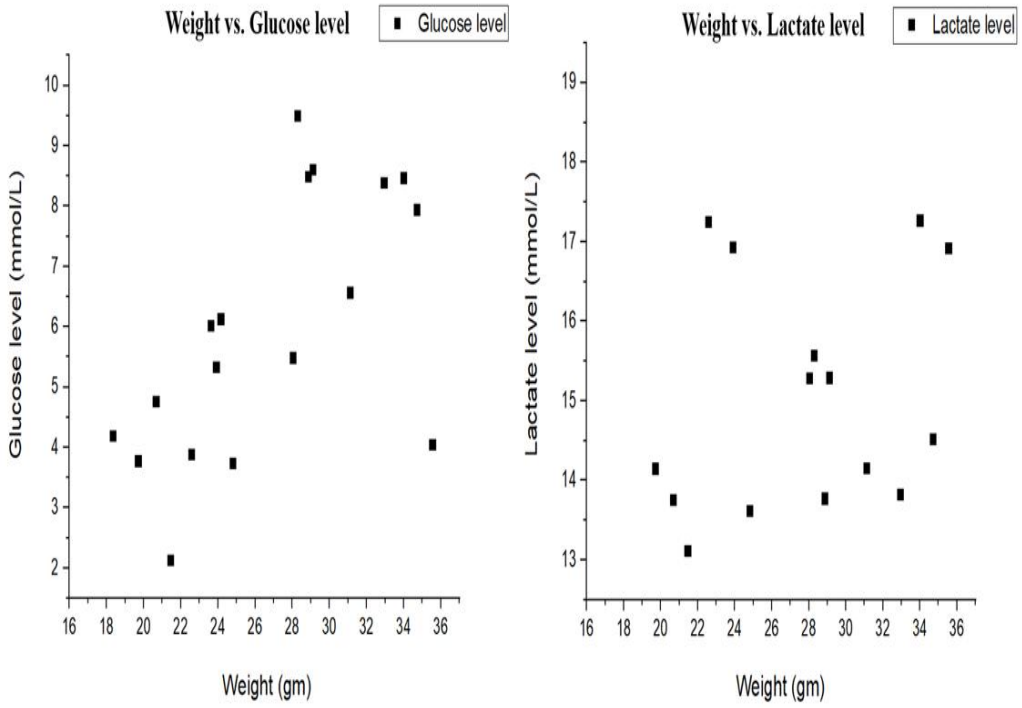

Figure 8. Glucose and lactate level of mice plotted against their body weight.

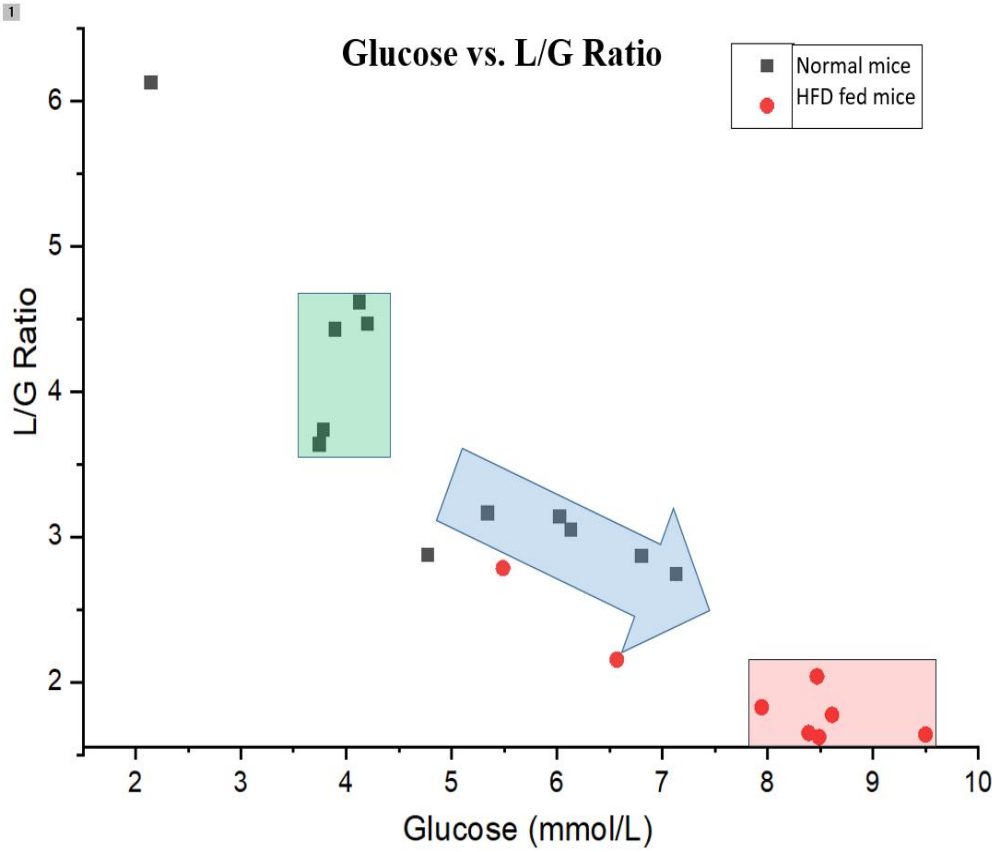

Figure 9. Blood plasma glucose level plotted against L/G ratio.

4. Discussion

Lactate and glucose are important metabolic component in human body and other animals. Despite of its functions in human health, lactate has been considered as the useless end product of glycolysis for decades. In our study, we measured glucose and lactate at the same time using the same kit material except their corresponding enzymes glucose oxidase and lactate oxidase respectively.

Lactate oxidase is unstable as compared to glucose oxidase due to which lactate assay has incubation time of only 5 minutes. The reaction buffer used also affected the stability of reaction cocktail prepared. Reaction cocktail prepared in potassium phosphate buffer were more stable as compared to sodium phosphate buffer. However, the exact reason is not found.

Significant increment in glucose levels were seen with increase in body weight of mice while little increase in lactate level were seen. It supports the answer to the finding of Daniel Klein that the ingestion of glucose alone has no any significant change in lactate level. However, the ingestion has delayed rise in blood pyruvate and significant increase in pyruvate and lactate level when administered with insulin [72]. It has also been stated that HFD can induce the metabolic disorders but does not affect lactate concentration in blood [70].

We also observed the significant increase in body weight of HFD fed C57Bl/6j mice than in normal chow fed mice. This mice strain is proved to be efficient in gaining weight compared to other mice strain [14, 73]. HFD fed mice group showed the increase in body weight significantly for 12 weeks of their age. However, slow weight

gain was seen afterward. Although, HFD fed mice continue to gain weight in significant amount. On the other hand, increase in weight gain of normal chow fed mice was almost constant.

In this study, an integrated procedure for glucose and lactate was developed using the single kit materials which makes the measurement of glucose and lactate faster and cheaper at the same time. Also, the L/G range of mice were found using the same protocol for healthy, transitional period and fat mice who might have chances of developing the obesity related diseases.

5. Summary

A highly sensitive amplex red based fluorescence assay for lactate were developed in this study. Plasma glucose and lactate concentrations of mice were measured using amplex red along with reaction catalyst HRP at the same time. Blood plasma glucose and lactate were measured in both 15 hours fasted normal chow fed mice and high fat diet fed mice. HFD fed mice gained the weight significantly within 1 week of HFD introduction. Also, the glucose levels were seen higher in the HFD fed mice than normal chow fed mice. Similarly, lactate level was found higher in HFD fed mice. Glucose versus L/G ratio was also plotted which showed with increase in glucose level decreases the L/G ratio.

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Optimization of Glucose and Lactate Assay in Blood Plasma for Health Monitoring

Seema Thapa
Department of Biomedical Engineering
Graduate School
Keimyung University
(Supervised by Professor Yun Seok Heo)

(Abstract)

Lactate has always been a neglected factor even though it is highly important for diagnosis and prognosis of critically ill patients. Lactate in such patients acts as an indicator of possible outcome, higher the lactate level in body more is the chances of mortality. Like glucose, lactate is also associated with type two diabetes. Obese diabetic patients have higher fasting plasma lactate level than the non-diabetic obese individuals. Likewise, diabetic patients have higher glucose level than the normal non-diabetic individuals. Glucose and lactate are not only related in diabetes but they both acts as an indicator in critically ill patients, as the hyperglycemia is also related to the mortality rate in critically ill patients in

hospital especially in patients with undiagnosed diabetes. Even though glucose and lactate are related to each other in many aspects, they are still studied separately. In our study, we measured glucose and lactate level in 15 hours fasted C57Bl/6j normal and high fat diet (HFD) fed mice following the same assay protocol using amplex red as substrate simultaneously. Glucose level was higher in HFD fed mice than normal chow fed mice. Similarly, the lactate level showed the same pattern. With increase in mice body weight, lactate level showed a gradual increase. Our study found relationship between lactate and glucose when different data were plotted. Among which, the plot of glucose with L/G ratio showed a significance to find out the transitional period in between normal and obese mice. This proves that our protocol can measure glucose and lactate at the same time using single kit which ultimately makes the measurement process fast, easy and economic.

건강 관리를 위한 혈장의 포도당과 젖산 분석의 최적화 연구

Thapa Seema
계 명 대 학 교 대 학 원
의 학 과, 의 용 공 학 과

지도교수 허 윤 석

(초록)

젖산염은 중증 환자의 예측과 진단에 매우 중요한 요소이지만, 심각하게 고려되지 않고 있다. 젖산염은 환자들의 건강상태를 나타내는 간접적인 지표로 활용되며, 신체의 젖산 수치가 높을수록 사망률이 더 높다. 포도당과 같이 젖산염도 제 2 형 당뇨병과 관련이 있다. 비만 당뇨병 환자는 당뇨가 아닌 비만 환자보다 혈장의 젖산염 수치가 높다. 포도당과 젖당류는 당뇨병뿐만 아니라 진단되지 않은 당뇨병 환자를 포함하여 병원 내 위독한 환자의 사망률과도 관련이 있으므로 응급 환자의 건강상태 지표 역할을 한다. 포도당과 젖산염은 서로 많은 연관이 있지만, 여전히 독립적으로 연구되고 있다. 본 연구에서 동일한 프로토콜에 따라 마우스(C57B1/6j)에 정상, 고지방 식이요법(HFD)을 동시에 제공한 후, 15 시간 이내에 포도당과 젖산염 수치를 측정하였다. 포도당 수치는 일반 먹이를 섭취한 쥐보다 HFD를 먹은 쥐에서 더 높았다. 마찬가지로 젖산염 수치도 유사한 패턴을 보였다. 쥐의 체중이 증가함에 따라 젖산염 수치는 점진적으로 증가하였다. 본

연구를 통해 젖산과 포도당 수치가 제시되었을 때, 두 물질의 관계를 발견하였다. 특히, L/G 비율이 있는 그래프에서 정상 쥐와 비만 쥐 사이의 과도기를 알아내는 데 의의를 보였다. 이번 연구를 통해 제시된 프로토콜은 궁극적으로 측정 과정을 빠르고, 쉽고, 경제적으로 만드는 단일 키트를 사용하여 포도당과 젖당을 동시에 측정할 수 있다는 것을 증명할 수 있다.