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Dyna Rochmyaningsih
Laboratory of Zoology, Bogor Agricultural University, Indonesia

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Abstract

Working memory is the ability in the brain to temporarily hold and process acquired information. To perform memory task, human brain needs sufficient glucose pertaining to its energy demand. Thus, fasting, as a temporary condition of no glucose intake into the body, would logically give negative effect on memory performance. This assumption is supported by the finding of untreated patients of hypoglicemia (low blood glucose) that has syndrome of confusion and may have trouble of concentrating. Previous study also showed that overnight and morning fasts produced slower memory recall on schoolchildren in United States and Peru and also for the college students in United Kingdom. It was proposed that metabolic stress and the importance of glucose in the making of acetylcholin were the reasons of the effects. To the contrary, previous study in Indonesia showed that daily fast did not influence the memory of college students but improve the memory performance of teenagers. Thus, following these adverse conclusions, this study aims to find out whether or not fasting influence memory performance of adult human. In this experiment, blood glucose concentration of each subject was electrochemically measured using glucometer while their visual working memory was tested using sequential Delayed Matching to Sample (DMS) task. In this task, subjects were asked to match a number of visual stimuli and the order of its appearance corresponding to the level of the test. To hold the memory of the stimulus, 1500 ms delay period was given in each trial. After 60 trials, data of the reaction time and accomplished level were extracted from the test and would be further analyzed using regression analysis. This test was done in the fasting and the non-fasting conditions for each subject. Result showed that blood glucose level were high around 2.5 hours (110-120 mg/dl), decreased within 6 hours, and then remained constant until 24 hours (80-100 mg/dl). This homeostasis phenomenon was suggested to be the reason why the fasting duration did not influence memory performances both in reaction time and accomplished level. Fasting duration did not significantly influence reaction time with P-value 0.438 in the non-fasting condition and 0.966 in the fasting condition ($P>0.05$). It also did not significantly influence accomplished level with P-value of 0.432 in the non-fasting condition and 0.642 in the fasting condition ($P>0.05$). This experiment did not record hunger which was suspected to affect attentional memory processes. Moreover, learning effect and habituation are more considered to affect working memory performance.

Introduction

Memory is the process by which people encode, store, and retrieve information. Encoding refers to the initial perception of the information, store refers to the creation of a permanent record of the encoded information, while retrieval refers to the use of information that has been stored. Memory is divided into two kinds: short term memory (STM) and long term memory (LTM). STM is the memory that will disappear in seconds or minutes while LTM is a memory that may last for days or decades (Peterson&Peterson 1959). In daily life, we use both of them in a system called working memory. Working memory is a system and process that holds and manipulates information temporarily in the brain (Baddeley 1996). In terms of temporal domain, working memory is a kind of STM. But it usually contains multiple STMs and manipulates them together at the same time for the complex mental processing.

In performing memory-related tasks, the brain needs sufficient glucose to meet its energy demand (Ragozzino *et al* 1996). The brain uses glucose almost exclusively for its energy needs and requires the continual supply of this sugar for normal function (Frissel 1990). Therefore, the lack of blood glucose has been suggested to correlate with memory performance (Benton & Parker 1998). This assumption is supported by the finding of untreated patients of hypoglicemia (low blood glucose) who have syndrome of confusion and may have trouble of concentrating (The National Institute of Diabetes and Digestive and Kidney Disease 2008).

In United Kingdom, Benton and Parker (1996) found that morning fasts did influence some aspects of memory of college students. In addition, Pollit *et al* (1998) found that overnight and morning fasts produced slower stimulus discrimination, increased errors, and slower memory calls, particularly in undernourished children. It was proposed that those alterations resulted from the state of metabolic stress which homeostatic systems works to maintain the glucose concentration (Pollit *et al* 1998). Furthermore, glucose consumption enhances the attentional processes in colleges and memory performance of Alzheimer and Down syndrome patients (Korol& Gold 1998). Initial explanation for this finding is that glucose is an important precursor in the making of acetyl-cholin, a neurotransmitter that is engaged in the process of memory consolidation (Ragozzino *et al* 1996).

Monday-Thursday fasting (MTFast) is a religious and cultural fasting practiced by observant Muslims from the dawn to the sunset with the condition of no food and liquid intake into the body. In Indonesia, certain communities do habitually MT-Fast and because of its geographical location, MTFast in Indonesia usually has 14 hours fasting duration. Hence, these people serve as good subjects through which this experiment can be performed.

Previous finding on this topics found that MTF did not influence memory task of college students in Bogor, Indonesia (Erlangga 2007). In addition, Octivano (2008) found that MTFast gives positive effect in working memory of teenager in Jakarta, Indonesia. Consequently, those adverse conclusions initiate a research to re-investigate how MTFast influences working memory on adult human in Bogor and Jakarta, Indonesia.

Methods and Materials

Materials and Equipments

Materials and equipments in this experiment covered those that were used in the glucose measurements and memory experiments, and also the questioners. Glucose measurements used blood lancets, lancing device, reagent strips that contains gold electrode, and glucometer. While the memory experiment used portable computer that has been installed with the computerized DMS, Sequential Delayed Matching to Sample (SPDMS). Furthermore, in order to get personal information of the subjects, questioners were used which include questions on their age, diabetic record, and last meal time.

Subjects and Procedure

Subjects in this research were 30 healthy adult human who were residing in Bogor, Tangerang, and Jakarta, Indonesia. They consisted of 14 males-16 females with an age of 20-48 years. All subjects habitually do MTFast except three of them that observed David Fast (David Fast is a habitual fasting on alternate days for the same fasting duration like MTFast).

In this experiment, each subject underwent the glucose measurements and memory tasks in two sessions (Fasting and Non-Fasting condition). In each session, subjects had their blood glucose concentration (mg/dl) measured five minutes before the experimental procedures. Both tests were done between 11.00 am – 04.00 pm. This range of time was due to the availibility of the subjects who were mostly workers. In addition to their physiological conditions, each subject had different last meal time and some took their supper before started their fasts.

Erlangga (2007) found that memory performance was influenced by learning ability of subjects. It was found that subjects gave better performances in second session than in the first session . Therefore, this experiment tried to minimize learning effect through crossover design. This design classified subjects into two groups (FN and NF). Group FN underwent the first session in the fasting condition and the second session in the non-fasting condition. While group NF underwent the first session in the non-fasting condition and the second session in the fasting condition.

Glucose Analysis

Blood glucose levels were measured using a glucometer (GlucoDr, All Medicus Co.Ltd, Gyeonggi-do, Korea). The device produces quantitative measurements of blood glucose level which is comparable to laboratory methods (Stewart 1976). A volume of 2.5-4 μL blood were extracted from the finger of the subject and then electrically analyzed using the glucometer.

DMS

Delayed Matching To Sample task (DMS) is a widely used method to study visual working memory (Elliot 1999). The subjects in the DMS test are asked to match visual stimuli. The mechanism of the DMS task went as the following stages. Firstly, one picture was presented on the center of computer screen for 1500 ms. After that, a delay period was given to subject to hold the memory of the stimulus. During this delay, a blank screen was displayed before the subjects for 1500 ms. Then, eight different pictures were presented on eight places, that is, every corner and every center of screen sides. Within 5000ms, subjects had to choose a picture that was same with the first stimulus.

Before doing the actual memory task (sequential DMS), subjects underwent a series of training tasks. Simple DMS test was used in this test in order to make subjects get acquainted with the technical side of the computerized test. This test has only one picture stimuli to be matched. The number of trials in training task averaged 10-15 trials.

The actual memory task used sequential DMS. It consisted of eight levels corresponding to the number of stimuli to be matched. A successive trial was made if subject matched the corrects stimuli in the right order of appearance. To go to the next level, each subject had to perform three successively correct trials. If they failed in one trial, they were downgraded one level. Each subject started from the first level and was allowed to perform 60 trials for one session. Every subject was tested in two sessions (fasting and no fasting). Visual example of the Sequential DMS is depicted in Figure 1.

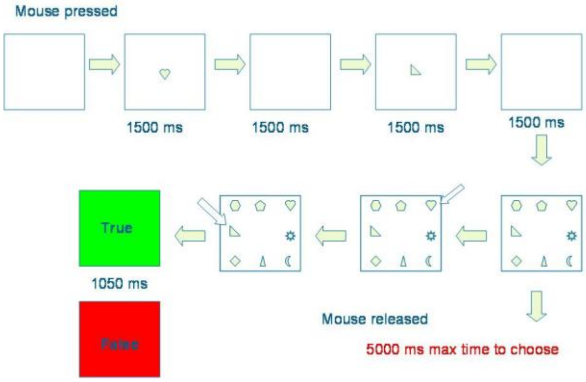

Figure 1. Sequential DMS task. A sequential DMS task was used as the memory task. In this task, subjects were asked to match the right stimuli in the right order of appearance. In this figure, a subject undergoes a level 2 of the sequential DMS task where there are two stimuli need to be matched. A correct trial was reached if the subject clicked the heart shape and then the triangle shape. Clicking the triangle shape before the heart shape was considered as a false answer.

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Working memory performances are measured by recording reaction time and accomplished level of each subject. Reaction time is defined as the mean reaction time of each trial averaged for the whole session. Accomplished level is the ratio between accumulated score attained by subject divided by maximum score in level 8 without any errors. The model of the experiment is shown in Figure 2.

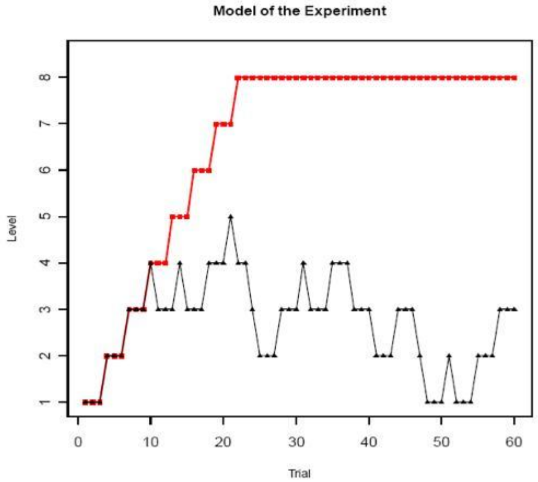

Figure 2. The Model of the Experiment. The figure represents the graphic extracted from the sequential DMS task done by each subject in one session. The task consists of 60 trials (abscissa) and eight levels (ordinate). Successive trials are represented by the black triangles and lines. The subject must perform three successive trials to go up to the next level and if he or she failed in one trial they would go down to the previous level. The red squares and lines represent the maximum virtual performance in the task. Maximum virtual performance is reached if the subject do successive trials consecutively without errors. After she or he attains level 8 then she or he is dismissed from the experiment and is recorded to have maximum scores. The basic calculation of accomplished level can be represented as the comparison of the whole area covered by the black lines to the whole area covered by the red lines.

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Statistical Analysis

Factors which are assumed to affect working memory are fasting and its duration, glucose level, and also sex. These factors were analyzed using regression analysis which is a part of Linear Model (Venables & Ripley 1999) implemented in *stat* package of the R program. Linear Model is a statistical approach to analyze relationship betweeen two variables as dependent variables (response) and independent variables (predictors) (Trochim 2006) while R program is an integrated suite of software facilities for data manipulation, calculation, and graphical displays (R Development Core Team 2007). Predictor in this experiment was fasting duration while responses were glucose level, reaction time, and accomplished level.

Results

Glucose Level

In Figure 3, correlation between glucose level and time duration after the last meal can be seen through the regression line in the non-fasting condition (Fig. 3a) and the fasting condition (Fig. 3b). Because the time of the last meal was different for each subject, note that the test was taken place within 7–24 hours after the last meal in the fasting condition, while in the non-fasting condition, the test was taken place within 6 hours after the last meal. In the non-fasting condition, glucose level seems to be decreased until 6 hours after the last meal while in fasting condition, glucose level tends to be constant until 24 hours. To examine this hypothesis, the data from both models were analyzed using Linear Model and the result of this analysis shown in Table 1. In making the statistical inference, standard error in the model was used to determined 95% confidence interval and α 5% (0.05) significance level. It can be seen from Table 1 that the Pr(>t) in the non-fasting condition is 0.0138 ($P<0.05$). Because this number is smaller than the significance level then it can be inferred that duration in the non-fasting condition significantly affect the level of blood glucose. While in the fasting condition, the Pr(>t) was 0.876 which was far bigger than the significance level ($P>0.05$), then it can be inferred that duration in the fasting condition did not affect the level of blood glucose.

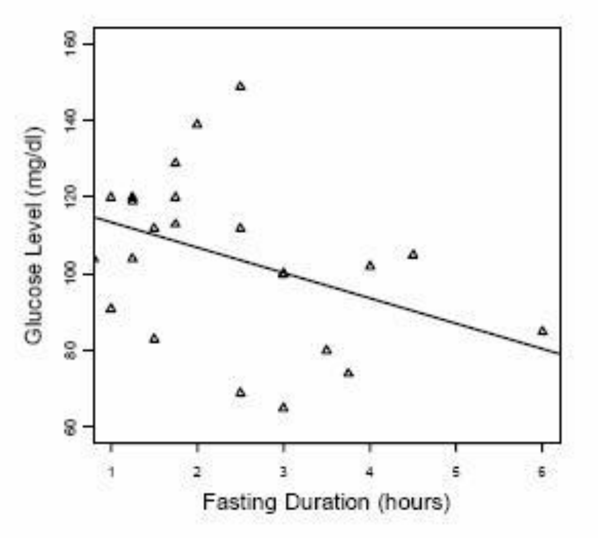

Figure 3a. Level of body's blood glucose after the last meal in non fasting condition (a) and fasting condition (b)

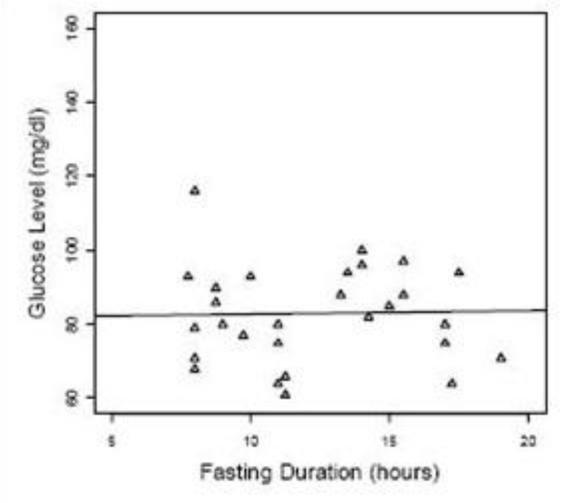

Figure 3b. Level of body's blood glucose after the last meal in non fasting condition (a) and fasting condition (b)

This statistical inference means the glucose level was high after the meal and decrease within 6 hours. Actually higher glucose level can be seen only during 2.5 hours from the last meal (110-120 mg/dl) and then decreased within 6 hrs (60-100 mg/dl). The graph was different in the fasting condition which had almost constant glucose level (around 60-100 mg/dl). So we can conclude that glucose level is pretty much constant after 6 hours from the last meal.

Table 1. Correlation between blood glucose level and fasting duration. In the non-fasting condition, estimated blood glucose level is around 120.130 mg/dl with probability value <2e-16 and the fasting duration in this condition affect

blood glucose level with probability value 0.0138 which was lower than significance level (5%).

Session		Estimate	Std. Error	t-value	Pr(>t)
No Fasting	Intercept	120.310	6.202	19.40	<2e-16***
	Dur.	-6.644	2.517	-2.64	0.0138
Fasting	Intercept	81.995	7.213	11.367	5.29e-12***
	Dur.	0.00876	0.5505	0.158	0.876

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Reaction Time

Figure 4 shows correlation between reaction time and time duration in female (F) and male (M) subjects in the first session. From the graph, it can be seen that there were two outliers (F9 and F21) that had slower reaction times than the others. These outliers were determined to be excluded because these were the data from subjects that were not acquainted with computer in their daily life. Eventhough they did the training task, these subjects still stated that they were not confidence with the tasks. Furthermore, the two outliers are suspected to correlate with the age of subjects which is considered to be older in the age range of the current subjects. Thus, by excluding two outliers (F9 and F21), it can be seen that reaction times of female and male subjects were not so different. The mean of reaction times of females was 1423 ms. It was not different statistically from males who had 1323 ms as the mean of reaction times.

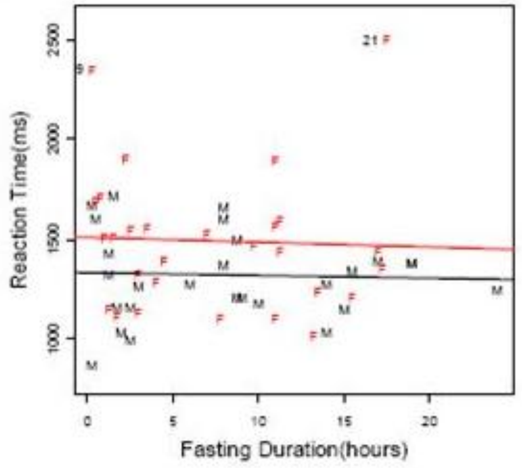

Figure 4 Reaction time correlated with time duration and sex difference

On the other hand, fasting duration alone did not affect reaction time both in the fasting and the non-fasting conditions (Figure 5). The linear analysis of this model can be read in

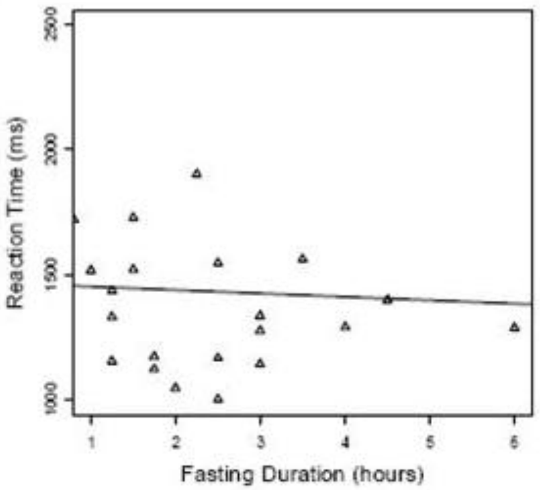

Figure 5a Reaction time correlated with time duration in non fasting condition (a) and fasting condition (b)

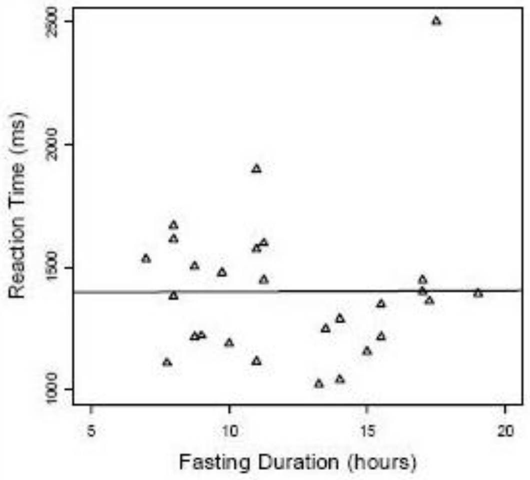

Figure 5b Reaction time correlated with time duration in non fasting condition (a) and fasting condition (b)

Table 2. In both models we can see that the regression lines were almost horizontal. The Pr(>t) in the non-fasting condition was 0.438 and 0.966 in the fasting condition. Both were above the significance level (0.05). This means that fasting duration both in the fasting and the non-fasting conditions did not influence reaction time.

Condition		Estimate	Std. Error	t-value	Pr(>t)
No Fasting	Intercept	1466.66	74.72	19.628	<2e-16***
	Dur.	-13.69	17.37	-0.788	0.438
Fasting	Intercept	1393.98	185.905	7.498	5.83e-08***
	Dur.	0.6103	14.0238	0.044	0.966

Table 2 Correlation between fasting and reaction time.

Accomplished level

Figure 6 shows the relation between accomplished level and time duration after the last meal. In the fasting condition (6a), it was observed that accomplished level of each subject were scattered around 20 to 50. The probability value (Pr) of this model is 0.432. This result means that the fasting condition (7-24 hrs after last meal) did not affect accomplished level of each subject. The same phenomenon was also observed in the non fasting condition (6b). The probability value of this model is 0.642 (Table 3). This result means that the non-fasting condition (0-6 hrs after last meal) also did not affect accomplished level.

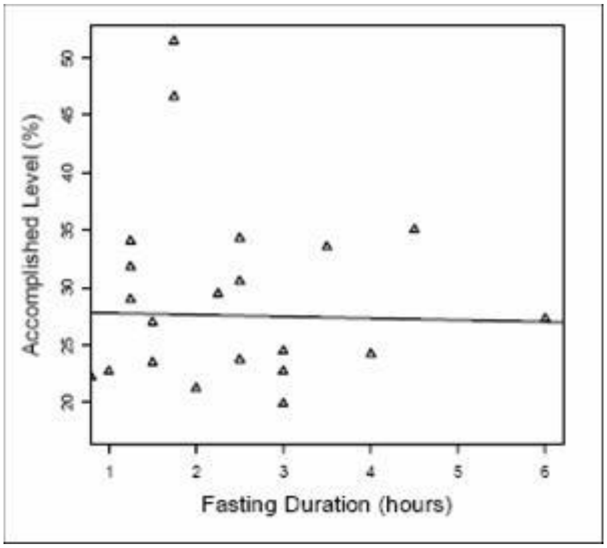

Figure 6a Accomplished level correlated with time duration in non-fasting condition (a) and in fasting condition (b)

Condition		Estimate	Std. Error	t-value	Pr(>t)
No Fasting	Intercept	28.567	1.903	15.01	6.43e-15***
	Dur.	-0.2116	0.4506	-0.47	0.642
Fasting	Intercept	33.5686	5.3654	6.256	9.2e-02***
	Dur.	-0.3262	0.4095	-0.797	0.432

Table 3 Correlation between fasting duration and accomplished level

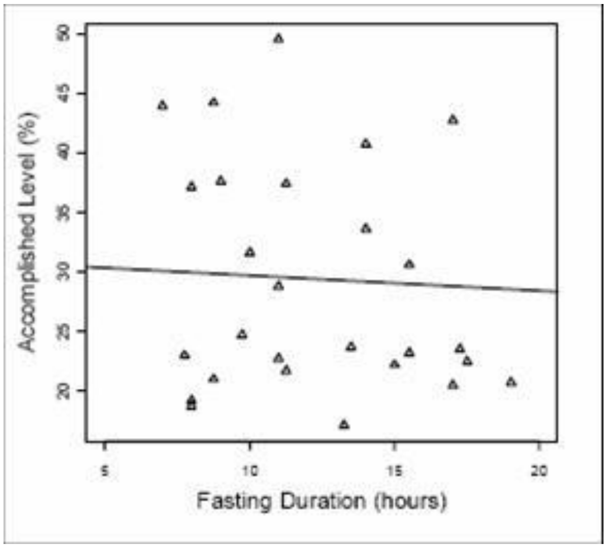

Figure 6b Accomplished level correlated with time duration in non-fasting condition (a) and in fasting condition (b)

Discussion

Result showed that glucose level remains constant after 6 hours of fasting. It negates the assumption that fasting means lack of glucose. In fact, even though there is no food supply in more than 5 hours (fasting condition), our body can still produce glucose from other sources inside. During the fasting conditions, the body's blood glucose needs are supplied by glycogen in the liver, although this is only enough for half a day. To continue to supply the body with glucose, sugars are generated from other metabolites, such as amino acids and triglycerides through a process called gluconeogenesis. This new synthesis of glucose can be derived from lactate, piruvate, citric acids cycle intermediets, and most amino acids (Voet&Voet 1995). Homeostasis is the way metabolic glucose levels are maintained consistently in the blood .

Working memory performance is not only affected by physiological factors. It is also affected by psychological factors. One of them is the sensation of hunger. Present research did not record hunger states of each subject. It is considered that hunger sensation affect working memory performance since it may distract attention of each subject. This is supported with previous findings that suggest hunger is associated with a bias in certain aspects of information processing which include attention in working memory system (Mogg *et al* 1998)

Another psychological factor is learning. Subjects have ability to associate visual stimulus with their knowledge and past experience (Malhotra 2003). For example they can associate one shape in DMS with a tree, bird, boat, so on and so forth. This associative learning can make subjects get better performance in the second session (Erlangga 2007). In this research, learning effect was minimized.by crossover design. This design made the data in fasting condtion comes from the first session (FN) and the second session (NF) and so with the non-fasting condition. Consequently, it can be assumed that learning has small effect on the result of the test. But even though it has been minimized, learning effect remains a factor in considering the accuracy of this analysis.

In addition, the gender of the subject is also assumed to affect working memory performance. In this research, there were two subjects who had longer reaction time in the first session. Both of them are female. Those outliers were determined to be excluded from the analysis because both subjects were not acquianted with computer in their daily life. Furthermore, the two outliers are suspected to correlate with the age of subjects which is considered to be older in the age range of the current subjects. So if those two outliers are excluded, it can be seen that reaction time of female were not so different from male. Thus, gender did not affect working memory performance in this study.

Another factor that also needs to be considered is habituation. This experiment used subjects who were habituated with daily fasting (MTFast) in their life. This habituation may be influence the adaptability of the body to deal with physiological stress that happens in the fasting condition.

Conclusion

In summary, fasting within 24 hours did not affect working memory performance of people who habitually fasted. Fasting duration did not influence memory performances both in reaction time and accomplished level. The homeostasis of the blood glucose level is suspected as the reason for this finding. In addition, learning process and habituation are more considered to affect it. Different observation may be expected for people who have no experience of fasting at all.

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