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Mixed effects of moderate exercise and subsequent various food ingestion on breath acetone

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Abstract

Acetone, which is exhaled with breath, is a by-product of lipolysis and could be used as a simple, useful indicator of lipolysis in the body because, unlike blood sampling, it can be measured non-invasively and repeatedly. Breath acetone concentration, however, is known to be affected by several factors such as exercise and food. We designed the experiments to evaluate the mixed effect on breath acetone of exercise and food ingestion in order to enhance the usefulness of breath acetone for monitoring fat loss. Seven healthy males performed moderate exercise for twice of 45 minutes with an interval of 15 minutes then rested for 4 hours. Exhaled air was sampled every 15 minutes throughout the experiment. The subjects took one of four types, sugar-rich, balanced, protein-rich and fat-rich, of food as lunch one hour after the exercises or kept fasting. In the case of fasting, breath acetone kept increasing significantly ($p<0.05$) compared with the rest value after the exercises until the end of the experiment. In contrast, in the case of taking any type of food, the change in breath acetone varied according to the food type. In the case of taking sugar-rich food, breath acetone significantly decreased ($p<0.05$) compared with the fasting case. This decrease might be due to a suppression of acetone production when carbohydrates such as sugar are supplied to a body in the fasting condition. In contrast, in the case of taking fat-rich food, breath acetone showed the higher level than the fasting case. This additional increase might be attributable to the promotion of ketone bodies production, including acetone, due to the ingestion of medium chain triglycerides (MCT) contained in the fat-rich food. We should therefore consider exercise and food ingestion in using breath acetone as a non-invasive indicator of lipolysis.

Keywords: breath acetone, lipolysis, exercise, food ingestion

1. Introduction

Obesity is a worldwide health issue due to its connection with lifestyle-related illnesses such as diabetes and hyperlipidemia [1]. Numerous methods to address obesity have been proposed, including diets [2] and exercises [3], yet

there is no royal road for losing weight. Regardless of the methods, many people trying to lose weight give up before reaching their goal. One reason for the abandonment of the methods is that it is difficult to know how much fat in one's body is burning at any one time. It is reasonable to assume that if people could identify and monitor fat burning in the body in real time, they could sustain motivation for the weight loss program and thereby achieve positive results.

We have examined acetone as an indicator of current fat burning in one's body. Acetone is a byproduct of lipolysis [4, 5]: Hepatic beta-oxidation of fatty acids leads to an accumulation of acetyl coenzyme A, which further divides into the ketone body acetoacetate. It undergoes decarboxylation and enzymatic degradation to acetone and beta-hydroxybutyrate, respectively. In contrast to beta-hydroxybutyrate and acetoacetate, acetone is highly volatile and, hence, it is exhaled in breath and excreted through urine. Breath acetone, therefore, could be expected to provide useful information about current fat burning in the body [6]. The comparative advantage of breath analysis over blood analysis is that breath can be sampled non-invasively, painlessly and frequently. There are, however, several problems to establish breath acetone as a non-invasive lipolysis indicator. Breath acetone concentration varies widely while the body is at rest [7, 8], and it is known to be affected by several factors such as exercise and food ingestion [6, 9, 10]. Few studies, however, have investigated mixed effects on breath acetone of moderate exercise and subsequent food ingestion, those acts often appear in our ordinary lives. For example, Bovey *et al.* [11] investigated the effects and reported that taking high carbohydeate food depressed breath acetone, however, in the experiment subjects took food intermittently and subsequently performed exercise. Güntner *et al.* [12] investigated the effects of exercise and subsequent glucose ingestion and reported that taking glucose after exercise decreased breath acetone, however, they fed subjects only glucose. The object of this research is unique because we focused on evaluating the mixed effects on breath acetone of moderate exercise and subsequent ingestion of various types of macronutrient such as sugar, protein and fat in order to enhance the usefulness of breath acetone for monitoring fat loss in our ordinary lives.

In addition, a current routine method for monitoring fat loss is using the respiratory quotient (RQ), however, to obtain reliable results of RQ, large amount of breath sample is required by an online gas monitor or with a douglas bag. Compared with the RQ measurement, the benefit of our breath aceotne method is requiring only one breath for one sample. To monitor breath acetone conveniently, inexpensive breath acetone sensors and portable breath acetone analyzers were developed for monitoring fat loss (*e.g.* [13,14]).

2. Method

Table 1. Physical properties of subjects

	Mean ± SD (n=7)
Age (year)	21.6 ± 1.9
Height (cm)	168.2 ± 4.7
Weight (kg)	58.4 ± 8.4
BMI (kg/m^2)	20.6 ± 2.7
Body fat percentage (%)	13.4 ± 5.1

2.1 Subjects

Seven healthy males were tested as subjects in this study. We could not recruit large number of subjects in this research, so we only recruited male subjects to reduce experimental factors unrelated to exercise and food. Before recruiting the subjects, we confirmed that no subject suffered from a metabolic disorder such as diabetes. Their profiles are summarized in Table 1. Body fat percentage was measured by the bioelectrical impedance (InnerScan50V, Tanita, Japan). Before participating in the experiments in this study, all subjects gave formal consent which was accepted by the ethics committee of the Research Institute of Environmental Medicine, Nagoya University (323-3).

2.2 Experimental design

Subjects visited the laboratory on six different days:

On day 1, the subjects performed preliminary exercise test using a bicycle ergometer. The test began at an initial power output of 90 watts, and the workload was increased 15 watts every minute until exhaustion. The pedaling rate was maintained at 60 rpm with the aid of a metronome. The highest $\dot{V}O_2$ value obtained during the test was determined as peak oxygen uptake ($\dot{V}O_{2peak}$).

On days 2-6, submaximal exercises and food ingestion were performed, the time course of the experiment is presented in Figure 1. Food ingestion or fasting before exercise affects substantially on breath acetone, that was reported in the previous articles [11, 15-17]. To control and unify the condition of food ingestion between the night before and the starting of the exercises among the subjects, they fasted from 23:00 on the day before each experiment, then took balanced food of 300 kcal at 8:00 as breakfast (Table 2). The experiment started at 9:30. Subjects took a rest for 30 minutes. At 10:00, they started exercise for 45 minutes using a bicycle ergometer. Exercise intensity was set at 40% $\dot{V}O_{2peak}$, because it was expected to maximize lipolysis according to the previous studies [18-20]. The mean ± the standard deviation of exercise intensities of all subjects were 72.4 ± 20.5 watts. After the exercise, they took a rest for 15 minutes, then repeated the same exercise for another 45 minutes. After these two periods of exercise totaling 90 minutes, subjects took a rest for 4 hours. They took lunch 60 minutes after the end of these exercises (12:45) or kept

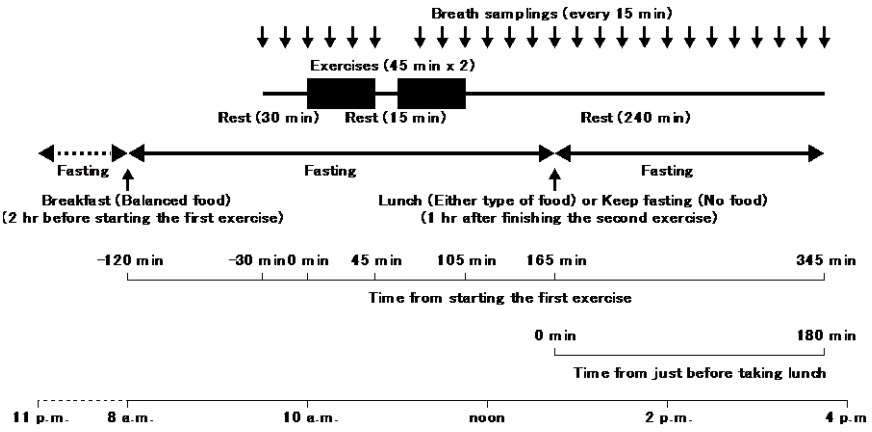

Figure 1. Experimental protocol of exercises, foods, and breath samplings.

fasting through this period. To compare the effect of food type on breath acetone, we prepared four types of food; sugar-rich, balanced, protein-rich, and fat-rich and served one of them in random order as lunch on each day. Namely, the same subject received a different diet or kept fasting on each day. The nutrition facts of each food are summarized in Table 2. We collected a total of 25 breath samples from subjects every 15 minutes from 9:30 to 15:45 except for 11:00. The reason why the exercise was separated into two parts and the breath sampling was skipped at 11:00 was the compatibility of the experimental condition with our previous experiments.

2.3 Breath analysis

The end-tidal air of subjects was collected using an exhaled air sampling kit (040906, BreathLab, Japan) consisting of a mouth piece, a discard bag (450 ml), and a collection bag (500 ml). The mouth piece and the discard bag was made of plastic and the collection bag was made of aluminum. Before each experiment, we flushed all collection bags out with pure nitrogen and made a blank test to ensure no analyte adsorption and sample contamination existed inside the collection bag. 500 µl of exhaled air was withdrawn from the collection bag using a gastight syringe (1750RN, Hamilton, U.S.A.) equipped with a reproducibility adapter (14725, Hamilton, U.S.A.). To remove the effect of acetone trapped in the water condensed inside the collection bag, the collection bag was preheated at 65°C, higher than the boiling temperature of acetone (56.5°C), by soaking the bag in a heated water bath before withdrawing the sample. Carbon dioxide was not controlled at the sampling. The measured sample was analyzed using a FID gas chromatograph (GC-4000, GL Science, Japan). The separation column in the gas chromatograph was a 2m-long stainless steel tube (inside diameter 2.17 mm) packed with adsorptive packing (Gaskuropack 54, 60/80 mesh, 2.2 g, GL Science, Japan). Pure nitrogen (99.9999%) was used as the carrier gas of the gas chromatograph and its flow rate was 33 ml/min. The injection port, the column oven, and the FID of the gas chromatograph were kept at 180°C steadily. The retention time of acetone was 1.7 minutes under these analytical conditions. No other peak which interfered with the acetone peak appeared around the retention time. The detection limit of acetone and the dynamic measurement range of the FID gaschromatograph detector was 0.05 ppm and 10,000, respectively. To calibrate the acetone concentration in each sample, we analyzed the standard gas which was the mixture of acetone (11.5 ppm) and pure nitrogen (residual) before and after each experiment.

2.4 Data analysis

Due to the limited number of the subjects (n = 7) in this research, the results are shown using median and quartile deviation instead of arithmetic mean and standard deviation,

Table 2. Nutrition facts of each food

	Breakfast	Lunch			
	Balanced food	Sugar-rich food	Balanced food	Protein-rich food	Fat-rich food
Brand name	CarolieMate	in Jelly Energy	CarolieMate	Whey Protein Pureisolate	Nisshin MCT Powder
Manufacturer*	Otsuka Pharmaceutical	Morinaga	Otsuka Pharmaceutical	Fine Lab	Nisshin Oilio
Intake (g)	60.0	180.0	40.0	20.0	13.0
Energy (kcal)**	300.0	180.0	200.0	72.8	100.0
Sugar (g)**	30.0	45.0	20.0	0.7	3.1
Fat (g)**	16.8	0.0	11.2	0.2	9.7
Protein (g)**	6.6	0.0	4.4	17.4	0.0

*All manufactureres are located in Japan.
**Amount per intake.

respectively, to reduce the effect of outliers. The median ± the quartile deviation of breath acetone concentrations at rest (10:00) in all experiments of all subjects were 0.91 ± 0.34 ppm. The concentration of breath acetone at rest is known to show wide individual differences [7, 8], although the concentration is not recognized to have systematic differences according to age and gender [21]. Therefore, it is inadequate to show the breath acetone results using absolute concentration. We instead monitored relative change in breath acetone concentration in the experiments. To remove individual differences, we defined the breath acetone ratio in terms of normalized relative change from a reference point in the experiment:

Breath acetone ratio = Breath acetone concentration / Breath acetone concentration at a reference point

Regarding the reference point, we set two points in the experiment: Just before starting the first exercise (10:00) to evaluate the whole variation in breath acetone through the experiment; just before taking lunch (12:45) to evaluate the effect on breath acetone of each food ingestion at lunch.

2.5 Statistical analysis

We checked the normality for each distribution by the Shapiro-Wilk's test and the homoscedasticity for two distributions in multiple comparison by the Bartlett's test. In the results of each test, not all distributions fulfilled the normality and/or the homoscedasticity. Therefore, we adopted a nonparametric method; the Wilcoxon's signed rank test for significant difference between two paired sample medians and the Steel's test for multiple comparison. We used "R" (version 4.2.1) in these statistical analyses.

3. Results

3.1 Breath acetone change by exercise

Figure 2 summarizes the variations of breath acetone ratio through the experiments for the four lunch types as well as the fasting condition. In the case of fasting, breath acetone ratio did not change much during two exercise periods. In contrast, the ratio almost kept increasing after these exercises until the end of the experiment. Significant increase ($p<0.05$) in the ratio was recognized compared with the ratio at rest by the Wilcoxon's signed rank test after 120 minutes until the end of the experiment (circles in Figure 2). Similar variations were recognized in the case of taking food at lunch; only in the case of taking sugar-rich food at lunch, the significant increase was disappeared after 240 minutes (Figure2 (b)).

3.2 Breath acetone change by the type of food

To elucidate the effect of taking each type of food on breath acetone, we recalculated the breath acetone ratio by resetting the reference point at the time just before taking lunch (165 minutes in Figure 2). Figure 3 summarizes the variations in the recalculated breath acetone ratio for the four lunch types as well as the fasting condition. The ratio after taking lunch more clearly varied according to the type of food compared with the fasting condition. In the case that subjects did not take lunch and keep fasting, the ratio almost kept increasing as mentioned above. In the case of taking sugar-rich food, the ratio gradually decreased until the end of the experiment. Significant decrease ($p<0.05$) in the ratio was recognized compared with the ratio of the fasting case at each time by the Steel's test after 45 minutes until the end of the experiment (circles in Figure 3 (b)). In the case of taking balanced food, the ratio also gradually decreased as the sugar-rich case. Significant decrease ($p<0.05$) in the ratio was recognized compared with the ratio of the fasting case at 60, 120 and 135 minutes (circles in Figure 3 (c)). In contrast, in the cases of taking fat-rich food or protein-rich food, significant increase or decrease was not recognized in the ratio compared with the ratio of the fasting case after taking lunch.

4. Discussion

4.1 Breath acetone change by exercise

Submaximal aerobic exercise has been recognized to promote lipolysis and increase breath acetone concentration [19]. In this research, the breath acetone ratio did not increase substantially during the moderate exercises but increased significantly ($p<0.05$) compared with the rest value after the exercises (Figure 2). That means there was a time lag between the exercises and the increase in the breath acetone ratio. Such a time lag was also reported in the previous studies [13, 22, 23]. Acetone is one of ketone bodies, and it has been recognized since the early twentieth century that ketone bodies contained in urine or blood increased not during exercise but after exercise, that was so called "post-exercise ketosis" [24-26]. Acetone as well as beta-hydroxybutyrate are mainly produced from free fatty acid via acetyl coenzyme A and acetoacetate in the liver, and this process does not proceed promptly by moderate exercises: Free fatty acid in blood increases during exercise, in contrast, acetone and beta-hydroxybutyrate in blood increase after exercise [22]. Although ketone bodies in blood were not analyzed in this research, the time lag between the exercises and the increase of breath acetone ratio is consistent with these previous results.

4.2 Breath acetone change by the type of food

4.2.1 Sugar-rich food. Breath acetone ratio gradually decreased after taking sugar-rich food for lunch compared with the fasting condition, and significant difference

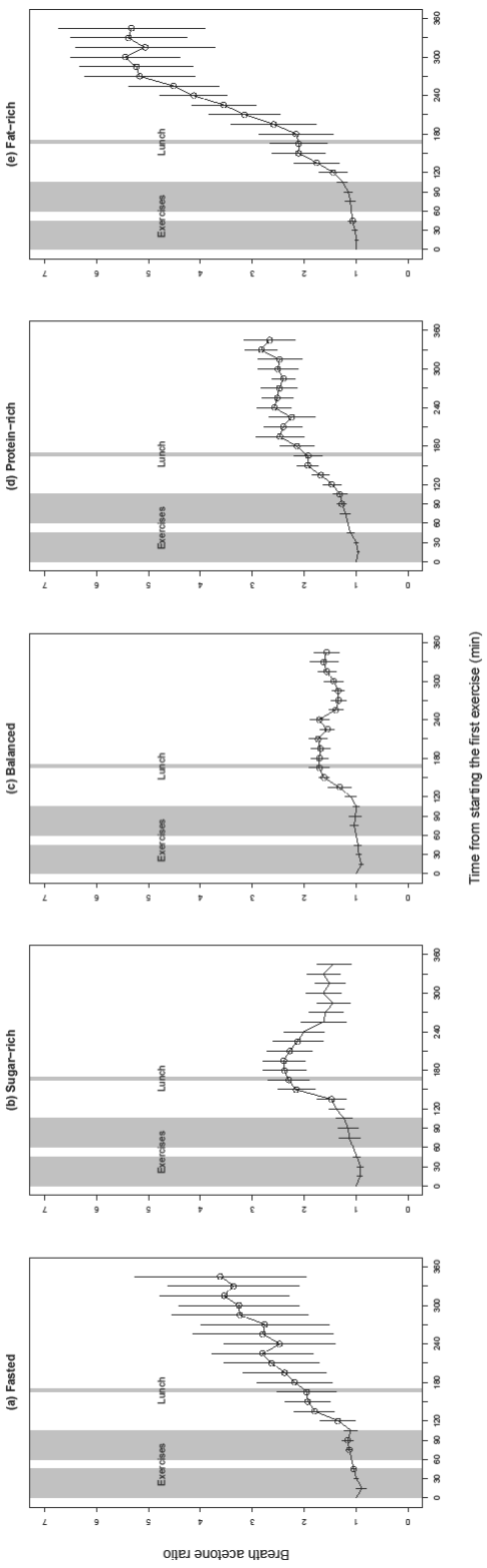

Figure 2. Variations in breath acetone ratio for fasting and four types of food through the experiment. Circles on each plot show the significant difference ($p<0.05$) compared with the rest value of each case.

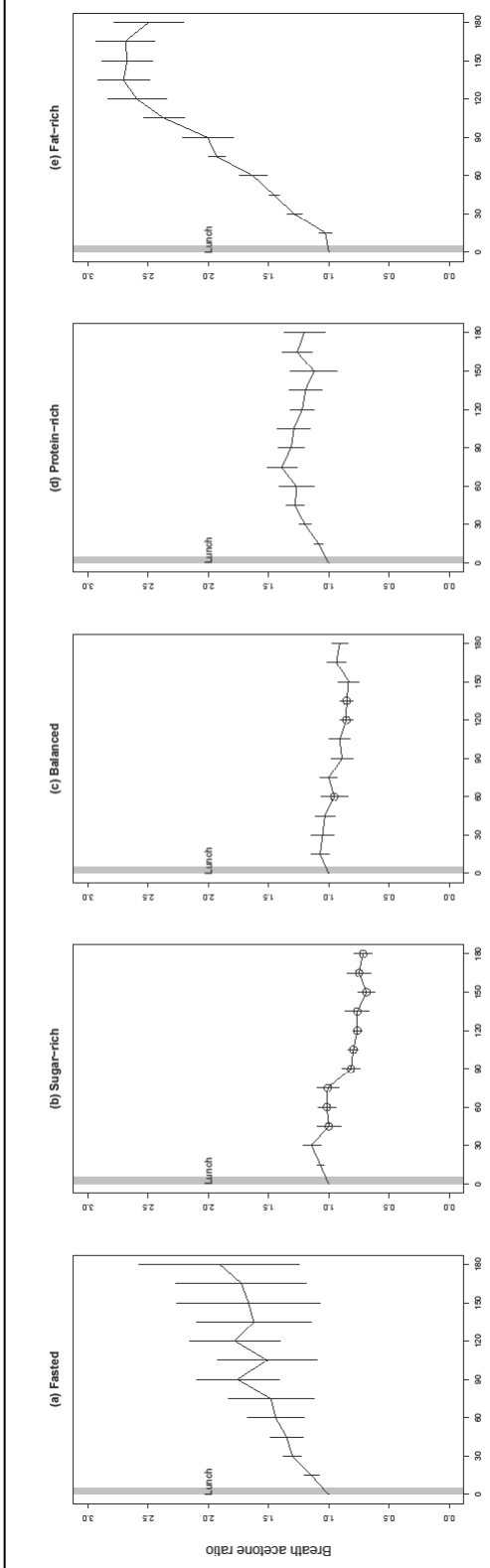

Figure 3. Variations in breath acetone ratio for fasting and four types of food after taking lunch. Circles on each plot show the significant difference ($p<0.05$) compared with the fasting case at each time.

($p<0.05$) was disappeared after 240 minutes in Figure 2 (b). On the other hand, significant difference ($p<0.05$) was recognized between fasting and taking sugar-rich food after 45 minutes in Figure 3 (b). The trend that breath acetone or total ketone bodies in blood once increased by fasting or exercise turned to decrease by the ingestion of high carbohydrate food was previously reported [11, 12, 26, 28, 29]. The production of ketone bodies including acetone is known to be promoted by starvation as substitute for carbohydrates [27], therefore, the ingestion of high carbohydrate (sugar-rich) food suppresses the starvation and decreases breath acetone as a result. The breath acetone ratio after taking sugar-rich food did not return to the rest value, 1, in these experiments. The similar trend was also reported by Güntner et al. [12]. We monitored breath acetone three hours after taking lunch and Güntner et al. [12] also monitored breath acetone three hours after taking glucose. If we monitor breath acetone longer than this research or if we increase the amount of food as we discuss below, the breath acetone ratio might return to the rest value.

4.2.2 Balanced food. Breath acetone ratio also gradually decreased after taking balanced food as in the case of taking sugar-rich food, and significant difference ($p<0.05$) was recognized several times between fasting and taking sugar-rich food (60, 120 and 135 minutes in Figure 3 (c)). The sugar contained in the balanced food might be responsible for the decrease of breath acetone after lunch as the ingestion of the sugar-rich food.

4.2.3 Protein-rich food. The median of the breath acetone ratio of taking protein-rich food showed lower value than that of the fasting case, although significant difference was not recognized after lunch between fasting and taking protein-rich food (Figure 3 (d)). The protein-rich food did not significantly decrease breath acetone ratio as the sugar-rich food but seemed to somewhat suppress acetone production promoted by exercise and fasting. This suppression might be due to the amino acid composition of proteins contained in the protein-rich food. Amino acids are generally divided into two types; glucogenic amino acids and ketogenic amino acids [30]. Glucogenic amino acids could be converted to glucose, thereby suppressing acetone production as in the case of taking sugar-rich food. The protein-rich food used in this research (20g) contained 18 amino acids; 3.3 g of ketogenic amino acids (leucine and lysine) and 14.1 g of glucogenic amino acids (the other 16 amino acids). The amount of glucogenic amino acids was four times larger than that of ketogenic amino acids. The glucose converted from the glucogenic amino acids could suppress acetone production after lunch. The amount of sugar (glucose) converted from the glucogenic amino acids contained in the protein-rich food, however, might be smaller than the amount of sugar contained in the sugar-rich food (Table 2). Hence the decrease of breath acetone ratio after lunch in the case of taking protein-rich food was not significant compared with the case of taking sugar-rich food.

4.2.4 Fat-rich food. The median of the breath acetone ratio of taking fat-rich food almost showed higher value than that of the fasting case, although significant difference was not recognized after lunch between fasting and taking fat-rich food (Figure 3 (e)). Ketogenic foods such as the fat-rich food served in this research or low carbohydrate foods generally increase breath acetone or blood ketone bodies [9, 13, 15, 25]. In addition, the fat-rich food served in this experiment mainly consisted of medium chain triglycerides (MCT). It was reported that MCT were rapidly digested and increased breath acetone [28]. MCT reduce the hepatic release of glucose and increase hepatic acetoacetate and beta-hydroxybutyrate release [31]. These features of MCT might give additional increase in the breath acetone ratio after the subjects took fat-rich food for lunch.

4.2.5 Duration and magnitude of the effect of food ingestion on breath acetone. In Figure 3, both the increase in breath acetone ratio by taking fat-rich food and the decrease by taking sugar-rich food seem to reach a plateau about 120 minutes after taking lunch. These trends suggest that the effect of each food ingestion on breath acetone lasted about 2 hours in this experiment. Freund and Weinsier [28] demonstrated that the duration and the magnitude of the effects on breath acetone, increase by taking fat (MCT) or decrease by taking sugar (sucrose), were proportional to the quantity of ingested fat or sugar, respectively. The calorie which the subjects took at lunch in this research (72.8 – 200 kcal, Table 2) was less than one tenth of the average daily calorie intake of men aged 20 to 29 years in Japan (2111 kcal) [32]. Therefore, if people take regular amount of food in their ordinary lives, the effect of food ingestion on breath acetone might be larger than the results of the experiments in this research.

4.2.6 Estimation of the power of statistical tests. The number of subjects (n=7) was not large in this research. Although we statistically analysed the results by nonparametric methods, we instead estimated the power of the statistical tests by parametric methods using R functions; *power.t.test* for the breath acetone change by exercise and *power.anova.test* for the breath acetone change by the type of food. We set the parameters of *power.t.test* as follows: *n* (number of subjects) = 7, *delta* (difference of average of the fasting case between 0 and 165 minutes in Figure 2) = 1.04, *sd* (standard deviation of the fasting case at 165 minutes in Figure 2) = 0.75, *sig.level* (significant level) = 0.05 (5%), *type* = "paired", then we calculated the function and got *power* (the power of the test) = 0.86 (86%). We set the

parameters of *power.anova.test* as follows: *n* (number of subjects) = 7, *groups* (number of cases) = 5, *between.var* (variance of averages of the cases at 180 minutes in Figure 3) = 0.545, *within.var* (variance of the fasting case at 180 minutes in Figure 3) = 0.789, between 0 and 165 minutes in Figure 2) = 1.04, *sig.level* (significant level) = 0.05 (5%), then we calculated the function and got *power* (the power of the test) = 0.92 (92%). These powers of both tests were not so low, however, if the number of subjects is more increased, these powers will be more improved; for example, the significant difference between the fat-rich case and the fasted case might be recognized in Figure 3.

5. Conclusion

Breath acetone increased by moderate exercise and fasting, that reflected the activation of lipolysis in the body. On the other hand, breath acetone changed according to the type of nutrition ingested after the exercise: Sugar decreased breath acetone, in contrast, some types of fat (MCT) promoted acetone production. Breath acetone could be a promising candidate as a non-invasive easy indicator of lipolysis in our body, however, we should consider exercise and food ingestion in breath acetone measurement for monitoring fat loss.

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Disclosure Statement

The authors decleare no competing interests.

Ethical Statement

This research was conducted in accordance with the principles embodied in the Declaration of Helsinki and in accordance with our local statutory requirements. All participants were not under 16 and they gave written informed consent to participate in this study. This research did not involve any identifiable human subject. This article did not rely on clinical trials.

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