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Acetone Response with Exercise Intensity

Tetsuo Ohkuwa[1,*], Toshiaki Funada[1] and Takao Tsuda[2]
[1]*Department of Materials Science and Engineering, Nagoya Institute of Technology, Showa-ku, Nagoya,*
[2]*Pico-Device Co., Nagoya Ikourenkei Incubator, Chikusa-ku, Nagoya, Japan*

1. Introduction

Blood samples are commonly used to explain the mechanism of metabolism during exercise and diagnosis of certain diseases. However, blood sampling is invasive and usually accompanied by problems such as loss of blood, emotional stress and discomfort for volunteers. The collection and analysis of expired air and skin gas has a number of advantages compared to that of blood, and can be performed by non-invasive and painless procedure. These non-invasive volatile gas monitoring is attractive since they can be repeated frequently without any risk and their sampling does not require skilled medical staff. Especially, skin gases which emanated from human skin which enables collection from hands, arms, fingers and other local areas of the human bodies (Tsuda et al. 2011). Volatile organic compounds can be produced anywhere in the body and may reflect physiologic or pathologic biochemical processes. These substrates are transported via the bloodstream and exhaled through the lung (Schubert et al. 2004). Analysis of volatile organic compounds enable the observation of biochemical process in the body in noninvasive manner.

It well known that free fatty acid and glucose are the major energy fuels under usual circumstances. Ketones such as β-hydroxybutyrate, acetoacetate and acetone are generated in the liver via decarboxylation of excess Acetyl-CoA (Miekisch et al. (2004), mainly from the oxidation of fatty acids, and are exported to peripheral tissues, such as the brain, heart, kidney and skeletal muscle for use as energy fuels (Mitchell et al. 1995; Laffel et al. 1999). Acetone is one of the most abundant compounds in human breath (Miekisch et al. 2004). Acetone is mainly generated from non-enzymatic, decarboxylation of acetoacetate (Laffel et al. 1999; Owen et al. 1982). Ketones in blood increased gradually with respect to the length of fasting periods in diabetics and obese people (Tassopoulos et al. 1969; Reichard et al. 1979). Owen et al. (1982) demonstrated that in diabetic patients, plasma acetone concentration is significantly related to breath acetone concentration. Acetone in expired air increased after high-fat ketogenic diet treatment (Musa-Veloso et al. 2002). The relationship between acetone concentration in plasma and breath has been well established (Naioth et al. 2002). Yamane et al. (2006) reported that skin gas acetone concentrations of patients with diabetes were significantly higher than those of the control subjects. Turner et al. (2008) demonstrated that there is a clear relationship between the level of breath acetone and

* Corresponding Author

acetone released from the skin. This result suggested that emission rates of acetone from skin can be used to estimate the blood acetone level. The acetone concentrations in both skin and breath gases are potentially useful as an indicator of β-oxidation of fatty acid and ketosis (Naitoh et al. 2002; Miekish et al. 2004; Musa-Veloso et al. 2002), but less is known as to the effects of exercise on acetone concentration of skin gas and exhaled acetone.

Senthilmohan et al. (2000) reported that running exercise increased breath acetone concentration compared to the basal level. However, in their study exercise intensity was not clarified. Sasaki et al. (2011) reported that acetone concentration in expired air increased during graded and prolonged exercise such as walking or running. We have reported that acetone concentrations emanated from skin gas and in expired air increased with high exercise intensities of cycle exercise (Yamai et al. 2009) and hand-grip exercise (Mori et al. 2008).

2. Methods

2.1 Procedure of exercise

The participants for hand-grip and cycle exercise were 7 and 8 healthy males, respectively. When this study was started, the first step was to ascertain the reproducibility of the results for skin gas acetone concentration test during hand-grip exercise. First the skin gas acetone was collected for four times in one participant. In hand-grip exercise, the participants performed a dynamic hand-grip exercise of three different types of exercise during 60 sec (Exercise 1, 2 and 3). Exercise 1 was performed at 20 kg with one contraction per two sec. Exercise 2 was 30kg with one contraction per three sec. Exercise 3 was 10kg with one contraction per sec. In cycle exercise, the workloads were 360 (1.0kg), 720 (2.0kg), and 990 (2.75kg) kgm/min, and each stage was 5 min in duration. A pedaling frequency of 60 rpm was maintained.

2.2 Collection of expired air and skin gases

The expired air was collected in Douglas bag, and skin gas was collected in the sampling bag at rest, during and after cycle and hand-grip exercises. In skin gas sampling, the right hand was cleaned, and wiped with paper before skin gas collection. After the hand was inserted into the sampling bag, which was fixed to the elbow (cycle exercise) or the middle of the upper arm (the center point between olecranon and acromion) (Fig 1-A; cycle exercise; Fig 1-B; hand-grip exercise). All the air in the bag was sucked out with a glass syringe; The gas in the bag was replaced with 600ml nitrogen gas. The skin gas collected for 1 min, and measured acetone concentration by gas chromatography.

2.3 Analytical procedure and conditions

The sampling gas was analyzed with a cold trap gas chromatographic system (Yamane et al. 2006; Nose et al. 2006). The sample was automatically sucked into the stainless-steel tube from loop cooled with liquid nitrogen. After the sample injection valve was rotated from the trapping position to the injection position, the trap tubing was heated directly to aid thermal desorption of the acetones in the sample. Acetone concentration in gas samples was detected by a gas chromatography with a flame ionization (FID).

The concentration of acetone in expired air and skin gases was measured using modified methods of Yamane et al. (2006). Acetone was detected with a flame ionization detector

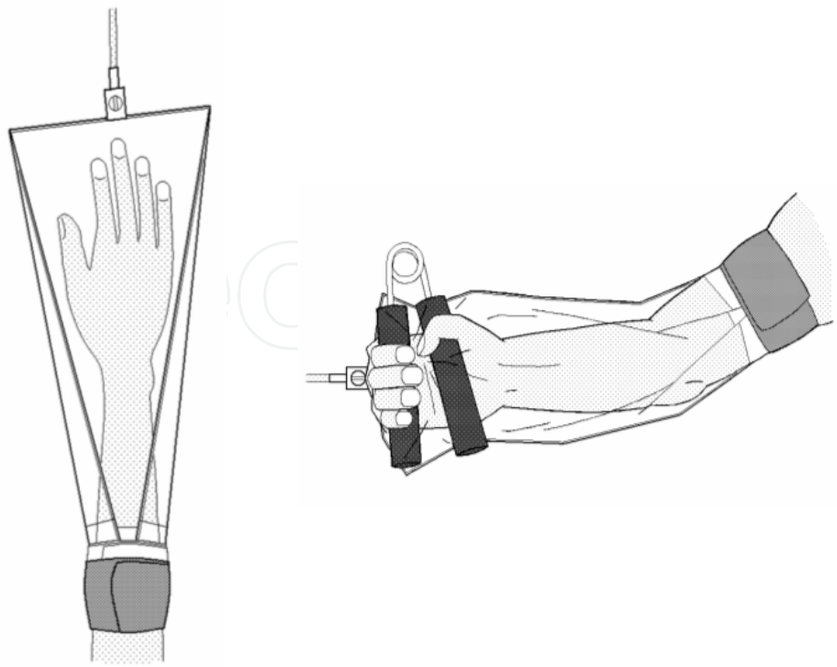

Fig. 1. Skin gas sampling methods (A: cycle exercise, B: hand-grip exercise).

(FID) by a Type GC-14B gas chromatograph (Shimadzu, Kyoto, Japan). The separation conditions for measuring the acetone were as follows: a Porapack Q capillary column (Type G-950: 1.2 mm internal diameter and 5.8 m long, Chemical Evaluation and Research Institute, Tokyo, Japan); the injection and detection temperature was 150℃; the column temperature was 100℃; and the retention time was 2.3 min.

3. Results

3.1 Hand-grip exercise

Table 1 demonstrated the mean (M) and standard deviation (S.D.) of skin gas acetone concentration at rest, during exercise, after exercise, and each coefficient of variation (C.V.) when one subject performed the same exercise four times. The C.V. of at rest, during exercise and recovery were 10.98, 9.52, 9.73, 8.67 and 8.49, respectively. Fig.2 demonstrates changes in acetone emanated from skin gas when one subject performed the same hand-grip exercise four times. The skin gas acetone concentration significantly increased during exercise compared to resting level, and decreased to almost the same as basal level immediately after 1.0-2.0 min during recovery.

	Rest	Exercise	Recovery		
			1.0-2.0 min	3.0-4.0 min	5.0-6.0 min
Mean (ppm)	0.082	0.147	0.113	0.105	0.106
SD	0.009	0.014	0.011	0.0091	0.009
C.V. (%)	10.98	9.52	9.73	8.67	8.49

Table 1. Mean, SD and CV of skin gas acetone concentration at rest, during exercise and recovery.

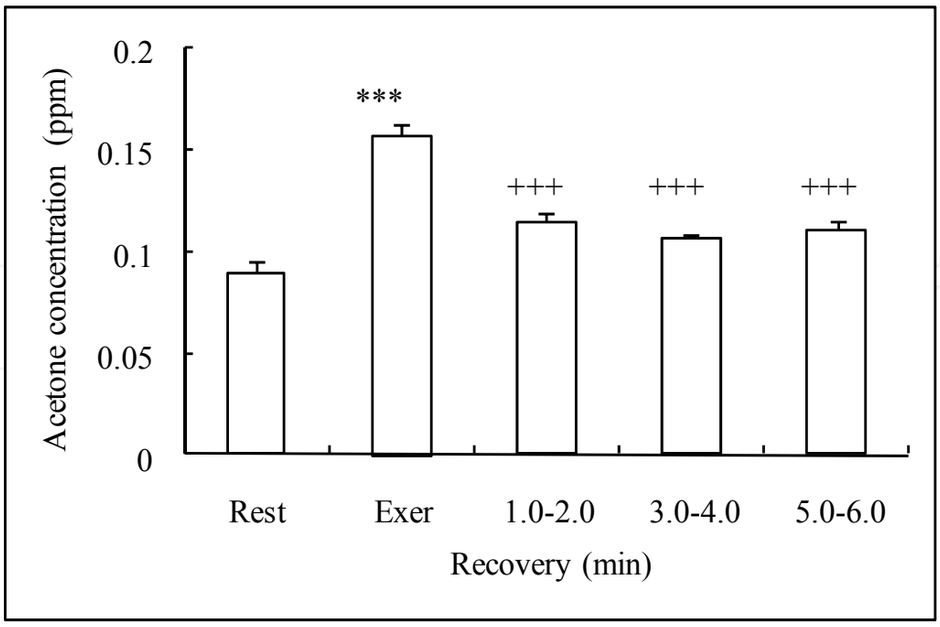

Fig. 2. Skin gas acetone concentration at rest, during hand-grip exercise and recovery. One subject performed the same hand-grip exercise four times. Values are M±SEM. **p<0.01, significant difference compared to rest ; ++p<0.01 significant difference compared to exercise.

Acetone concentration in skin gas during hand-grip exercise 2 (30kg ×20 times) was significantly higher than basal level (Figure 3). Although skin gas acetone during exercise 1 (20kg×30 times) and exercise 3 (10kg×60 times) increased, significant difference was not found. No significant difference was found in skin gas acetone concentration during hand-grip exercise among exercise 1, 2, and 3.

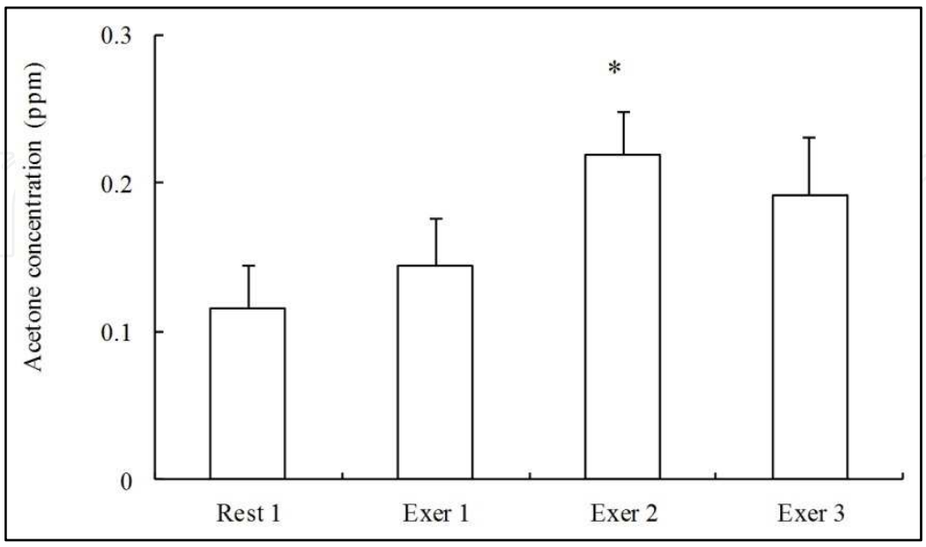

Fig. 3. Comparison of skin-gas acetone concentration among exercise 1, 2 and 3. Values are M±SEM. *p<0.05, significant difference compared to rest.

3.2 Cycle exercise

Figure 4 represents the changes in acetone concentration in expired air at the basal level, 360, 720 and 990 kgm/min during cycle exercise and recovery of 5 min after the 990 kgm/min exercise. Acetone concentration of expired air tend to increase with an increase in exercise intensity. The acetone concentration of expired air at 990 kgm/min significantly increased compared with the basal level ($p<0.05$). Figure 5 shows the changes in acetone excretion in expired air at the basal level, during cycle exercise and recovery. The acetone

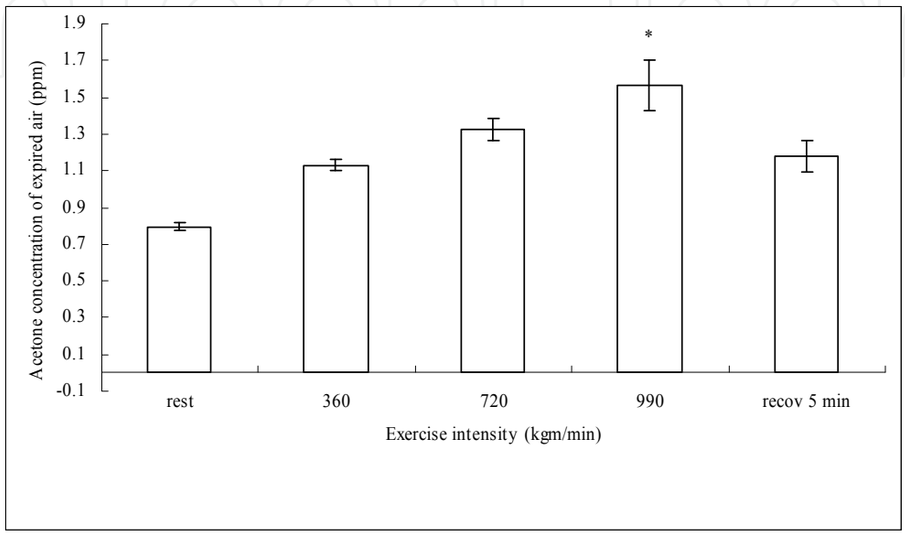

Fig. 4. Changes in acetone concentration in expired air at the basal level, during cycle exercise and recovery.*$p<0.05$ significant difference compared to rest.

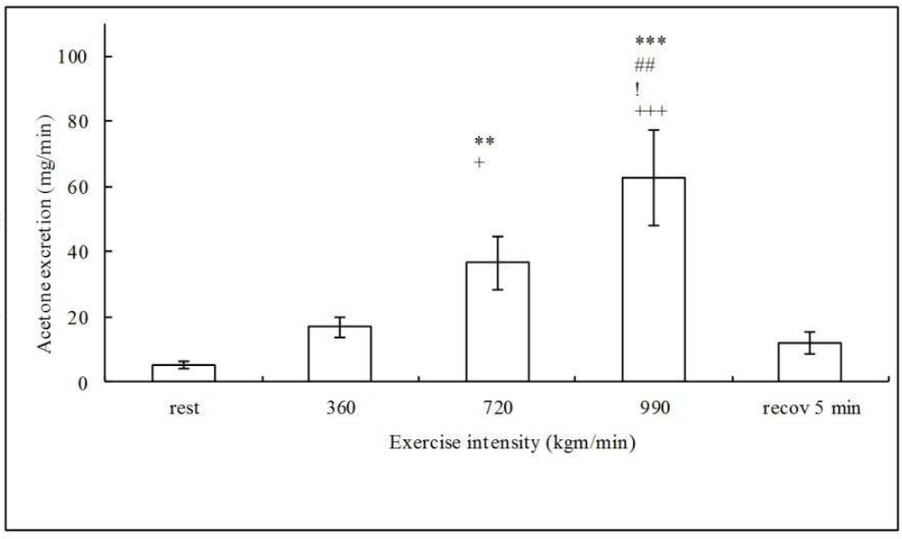

Fig. 5. Changes in acetone excretion in expired air at the basal level, during exercise and recovery. *** $p<0.001$, **$p<0.01$ significant difference compared with resting value. ## $p<0.01$ significant difference compared to 360 kgm/min, ! $p<0.05$ significant difference compared to 720 kgm/min, +++ $p<0.001$, + $p<0.05$ significant difference compared to 5 min after exercise.

excretion at 720 and 990 kgm/min significantly increased compared with the basal level ($p<0.05$). As shown in Figure 6, the skin gas acetone concentration at 990 kgm/min significantly increased compared with the basal level and 360 kgm/min ($p<0.05$). Acetone concentration in expired air was 4-fold greater than skin gas at rest and 3-fold greater during exercise ($p<0.01$). There is a significant relationship between skin gas acetone concentration with expired air ($r=0.752$, $p<0.01$, Fig.7).

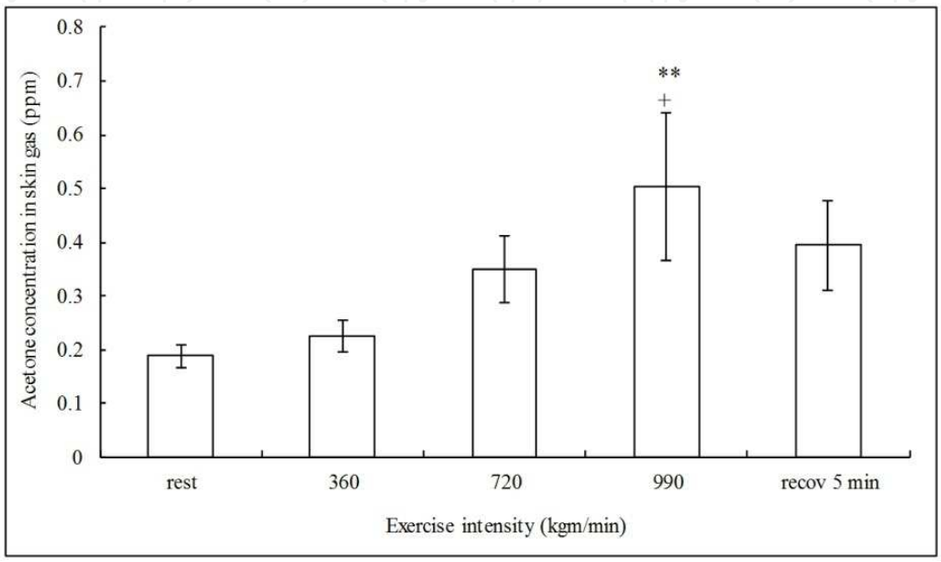

Fig. 6. Changes in skin gas acetone concentration at the basal level, during exercise and recovery. ** $p<0.01$ significant difference compared to rest. # $p<0.05$ significant difference compared to 360kgm/min.

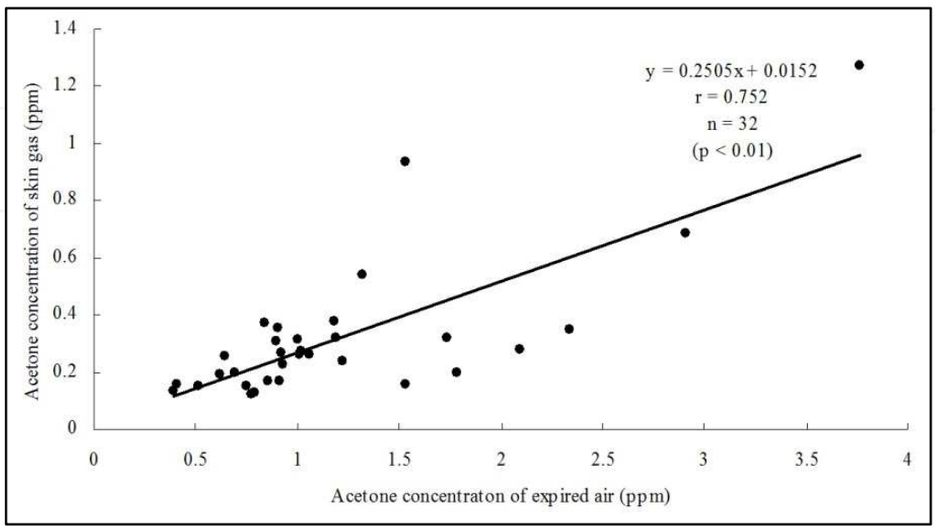

Fig. 7. The relationship in acetone concentration between in expired air and skin gas.

4. Discussion

Acetone is drived from spontaneous, non-enzymatic, and decarboxylation of acetoacetate when there is an insufficient supply of blood glucose (Owen et al. 1982). The relationship between acetone concentration in blood and breath has been well established (Owen et al. 1982). Previously, it has been demonstrated that skin gas acetone is correlated with breath acetone (Naitoh et al. 2002). Yamane et al (2006) also reported that skin acetone concentrations of patients with diabetes were significantly higher than those of the control subject. Naito al. (2002) reported that concentrations of acetone in both skin gas and breath increased according to the length of fasting periods, and also demonstrated that there is a good relationship between skin and breath acetone concentration.

It is a well known fact that ketogenesis is enhanced in diabetes, fasting and exercise (Féry et al. 1983; Balasse and Féry (1989); Wahren et al. 1984). Balasse and Féry (1989) reported that plasma ketone bodies increased after treadmill running at the intensity of 40% ~ 60% maximal aerobic capacity for 120 min compared with before exercise, and also observed that the plasma ketone bodies was higher in 60% maximal power compared to that of 40 % maximal power.

The C.V. of skin gas acetone concentration during hand-grip exercise was 9.52, when skin gas acetone measured four times in one subject. These results indicate that reasonable reproducibility is obtained in measurements of skin gas acetone concentration during hand-grip exercise and that determination of skin gas acetone is highly reliable.

The Exercise 2 of the hand-grip exercise (30kg ×20 times) showed an increase in acetone level of skin gas compared to basal level. The exercise 1 (20kg×30 times) and 3 (10kg×60 times) tend to increase skin gas acetone, but the increase was not significant. Although the total work is the same between Exercise 1,2 and 3, acetone concentration in Exercise 2 was increased compared to rest. The results of our study coincidently is comparable with the result from previous study (Balasse and Féry 1989). These experiments suggest that acetone concentration in blood and skin gas are related to intensity of exercise.

Senthilmohan et al. (2000) have demonstrated that acetone concentration of expired air increased in running exercise (Senthilmohan et al. 2000), but was not clear whether the acetone concentrations of skin gas increased with an elevation of exercise intensity. Acetone concentration of expired air and skin gas at the intensity of 990kgm/min has significantly increased compared to the basal level (Fig. 5 and 6). These results basically agreed with those of other studies (Balasse and Féry 1989; Féry et al.1983; Féry et al.1986). Sasaki et al. (2011) demonstrated in the graded exercise test that acetone level in exhaled air began to increase at the intensity of 39.6 % of maximal oxygen uptake. From these results, it is clear that acetone concentration of expired and skin gases increased with elevation of exercise intensity.

FFA and ketone bodies in blood increase during prolonged exercise and increase in mobilization results in elevation of utilization (Loy et al. 1986; Ravussin et al. 1986; Sasaki et al. 2011). The acetone concentration correlates with the concentration of blood ß-hydroxybutyrate (Reichard et al. 1979; Yamane et al. 2006). The kinetic responses of ketone bodies to exercise is very complex. The increase in ketogenesis is related to the rise in FFA

levels and to an increase in the ketogenic capacity of the liver (Balasse and Féry 1989). Exercise is known to increase the hepatic conversion of FFA to ketones in control group (Wahren et al. 1984). However, Wahren et al. (1975) have shown that uptake of ketone bodies by the leg muscle was of minor importance during bicycle exercise in control subject. Wahren et al. (1984) suggested that the rate of hepatic ketogenesis can be influenced by the rate of lipolysis (FFA release from adipose tissue) and the blood flow to the liver, and by the fractional extraction of FFA by the liver and an increase in the proportion of FFA converted to ketones. Furthermore, the increase of plasma catecholamines in response to the exercise may have contributed to the augmented fractional extraction of FFA by the splanchnic bed, which was observed in the control in response to exercise (Wahren et al. 1984). Dynamic hand-grip exercise increased norepinephrine (Costa et al. 2001). The increase of skin gas acetone might have been caused by norepinephrine secretion, which was increased during dynamic hand-grip exercise.

5. Acknowledgement

It was concluded in this report that acetone concentration in expired air and skin gas is directly related to exercise intensity. This human skin gas project was supported by the Aichi Science and Technology Foundation.

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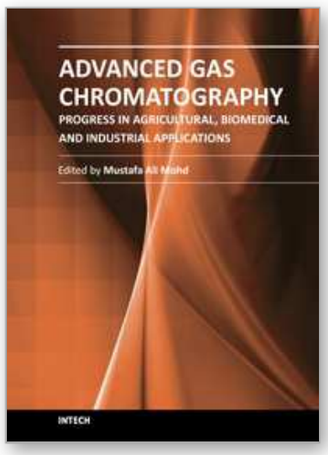

Advanced Gas Chromatography - Progress in Agricultural, Biomedical and Industrial Applications

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Progress in agricultural, biomedical and industrial applications' is a compilation of recent advances and developments in gas chromatography and its applications. The chapters cover various aspects of applications ranging from basic biological, biomedical applications to industrial applications. Book chapters analyze new developments in chromatographic columns, microextraction techniques, derivatisation techniques and pyrolysis techniques. The book also includes several aspects of basic chromatography techniques and is suitable for both young and advanced chromatographers. It includes some new developments in chromatography such as multidimensional chromatography, inverse chromatography and some discussions on two-dimensional chromatography. The topics covered include analysis of volatiles, toxicants, indoor air, petroleum hydrocarbons, organometallic compounds and natural products. The chapters were written by experts from various fields and clearly assisted by simple diagrams and tables. This book is highly recommended for chemists as well as non-chemists working in gas chromatography.

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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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