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Acetone and 6-Methyl-5-Hepten-2-One in Skin Gas Increase during Handgrip Exercise

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Abstract: The present study investigated the effect of dynamic handgrip exercise on acetone, 6-methyl-5-hepten-2-one, acetaldehyde, and hexanal levels in skin gas. Participants in this experiment consisted of six healthy males. Skin gas was collected two times. First, skin gas during rest (i.e., before the exercise) was collected for 3 min into the sampling bag. Next, the exercise using a handgrip exercise instrument was performed. The hand performed a dynamic handgrip exercise for 3 min inside the sampling bag, exerting one 30 kg contraction per second. The blood flow at the end of the handgrip exercise increased by about 1.4 times that before exercise and then decreased to basal levels immediately thereafter. Acetone and 6-methyl-5-hepten-2-one concentrations after exercise significantly increased relative to basal levels. Significant differences were not observed in acetaldehyde and hexanal concentrations between at rest and after handgrip exercise. The amount of acetone and 6-methyl-5-hepten-2-one released from forearm skin increased during dynamic handgrip exercise. Acetaldehyde and hexanal levels did not increase during exercise compared with levels at rest.

Keywords: 6-Methyl-5-Hepten-2-One, Acetone, Acetaldehyde, Hexanal, Human Skin Gas

Introduction

Several hundreds of volatile organic compounds (VOCs) are released through breath and skin in healthy subjects (de Lacy Costello et al., 2014). VOCs in exhaled breath and skin samples may be considered as a non-invasive biochemical index for judging normal from diseased states (Mochalski et al., 2014[a]). VOCs from skin are clinically significant (Turner et al., 2006), as their patterns directly relate to the physiological status of an individual (Mochalski et al., 2014[b]). One study has demonstrated that the ketones (acetone and 6-methyl-5-hepten-2-one), as well as the aldehydes (acetaldehyde and hexanal) occur in high

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concentrations in gases released from the skin (Mochalski et al., 2014[c]).

We previously demonstrated that skin-gas acetone concentrations in healthy subjects during dynamic handgrip exercises and during cycling increase gradually according to exercise intensities (Mori et al., 2008; Yamai et al., 2009). Handgrip exercise is known to elevate the brachial-artery diameter, muscle oxygen uptake, and brachial-artery mean blood flow (MacDonald et al., 2001). Acetone is produced in large amounts in the liver during spontaneous decarboxylation of acetoacetate (Schwarz et al., 2009) and is formed in human skin during reactive oxygen species (ROS)-induced degradation of squalene (Petrick et al., 2009). The human skin surface contains lipids such as cholesterol, triglycerides, squalene, and fatty acids (Smith et al., 2008). However, no other reports on the effects of dynamic handgrip exercise on skin VOCs, except acetone, have been published.

6-Methyl-5-hepten-2-one is also produced in human skin during ROS-induced degradation of squalene (Fruekilde et al., 1998). Acetaldehyde has been found to be formed by oxidative degradation of linolenic acid (Fujisaki et al., 2002) and by ethanol metabolism (Crabb et al., 2004). Moreover, hexanal is produced from linoleic acids (Wisthaler & Weschler 2010; Frankel 1999). The present study, therefore, investigated the effect of dynamic handgrip exercise on acetone, 6-methyl-5-hepten-2-one, acetaldehyde, and hexanal levels in skin gas.

Subjects and Methods

Subjects

Participants in this experiment consisted of six healthy males. Their ages ranged from 22 to 60 years (mean ± SD = 30.2 ± 14.8 years). They were in good health, were not taking any medication, and were nonsmokers. The purpose of the study, as well as the procedures and possible risks, was fully explained to each subject before a signed document of their informed consent was obtained. The study was approved by the Ethical Committee of the Nagoya Institute of Technology.

Experimental methods

All participants had breakfast at least 3 hour before experiment. All experiments were performed after the participants had rested for 20 min. The room temperature was set at 25°C. A bag made of flexible PVDF sheet (DuPont, USA) was used for sampling (Fig. 1). Hands of participants were washed with running tap water for 30 sec and then with distilled water, and finally wiped with a paper towel (Kimwiper, Japan). Each hand was then inserted into the sampling bag, which was fixed to the middle of the upper arm (the point between the olecranon and acromion) with a sealing film (Parafilm M, USA). The gas in the bag was replaced with 100 ml of nitrogen gas.

Skin gas was collected two times. First, skin gas during rest (i.e., before the exercise) was collected for 3 min into the sampling bag, and 50 ml of the gas was then drawn into a glass syringe. Next, the exercise using a handgrip exercise instrument was performed. The hand performed a dynamic handgrip exercise for 3 min inside the sampling bag, exerting one 30 kg contraction per second. Meanwhile, skin gas was collected into the sampling bag and then drawn into a glass syringe until 50 ml was collected prior to measurement.

Analytical conditions

It was measured by modified gas chromatography (GC) equipped with a flame ionization detector

(GC-2010, Shimadzu, Japan), a constant-temperature oven (CTO-20A, Shimadzu, Japan), a gas-trapping system (NIT-3, Pico-device, Japan) and a temperature controller (E5LD, Omron, Japan) for the gas line. The glass syringe filled with skin gas was brought to constant temperature (80°C) in the oven. After the temperature was allowed to stabilize, the gas was heated for 10 min and then analyzed. The gas injection rate was 30 ml. A DB-WAXETR (30 m, 0.32 mm I.D., 1.0 μm film, Agilent Technologies J&W, USA) GC column was used. The column temperature program was 40°C for 3 min, 20°C/min for 8 min, and 200°C for 5 min (total of 16 min). Helium was used as the carrier gas and makeup gas. Flow rates of the carrier gas, makeup gas, hydrogen gas, and air were 12.0, 20.0, 25.0, and 250.0 ml/min, respectively. The analytical curve for the standard specimen was constructed by using measurements on acetaldehyde (90.0%, Wako, Japan), acetone (99.5%, Kanto Chemical Company, Japan), hexanal (95.0%, Wako, Japan), and 6-methyl-5-hepten-2-one (98.0%, Tokyo Chemical Industries Co. Ltd., Japan). A blood-flow-volume meter was used to measure the blood flow volume (Omega Flo, FLO-C1, Omega Wave, Tokyo, Japan).

Statistical analysis

Values are presented as mean ± SEM. Data were analyzed by one-way analysis of variance with repeated measurements, with $p < 0.05$ determined to be statistically significant. When differences were obtained, post hoc analysis was performed by using Fisher's PLSD. Statistical analysis was performed with StatView software (v. 5.0, Avaccus Concept Inc.)

Results

Figure 2 shows changes in blood flow in the middle finger of one subject at rest and during the 3 min dynamic handgrip exercise. The blood flow increased with elapsed exercise time. The blood flow at the end of the handgrip exercise increased by about 1.4 times that before exercise and then decreased to basal levels immediately thereafter. Acetone and 6-methyl-5-hepten-2-one concentrations after exercise significantly increased relative to basal levels ($p < 0.05$, Figs. 3 and 4). Significant differences were not observed in acetaldehyde and hexanal concentrations between at rest and after handgrip exercise (Figs. 5 and 6).

Discussion

In this study, acetone levels in skin gas increased after handgrip exercise compared with basal levels. Results of this study agree with those in our previous study (Mori et al., 2008), which showed that acetone released from the skin after handgrip exercise at 60% maximal power increased in concentration compared with levels at rest. Acetone concentration in exhaled air and skin gas increased during moderate- and/or high-intensity exercise (Yamai et al., 2009). It is well known that plasma ketone bodies (acetoacetate, 3-hydroxybutyrate, and acetone) are formed mainly via oxidation of fatty acids in the liver and are exported to peripheral tissues (brain, heart, kidney, and skeletal muscle) for use as energy fuels (Laffel 1999).

Levels of ketone bodies are influenced by hepatic production of ketones and by extraction by working muscles and brain (Balasse & Ferry 1989; Wahren et al., 1975).

Acetone is derived from decarboxylation of acetoacetate (Owen & Trapp 1982). A previous study suggests that the rate of hepatic ketogenesis is affected by lipolysis, by blood flow to the liver, and by fractional extraction of free fatty acids converted to ketones (Wahren et al., 1975). Another study reports that acetone levels in exhaled air or blood increases during graded bicycle exercise at an intensity of 34.3% of maximal oxygen consumption (Sasaki et al., 2011; Wahren et al., 1975). In the present study, the intensity of the dynamic handgrip exercise for 3 min at 30 kg with one contraction per second was very high (it resulted in exhaustion in most cases). The increase in acetone levels in skin gas in our study might be due to increased lipolytic activity and levels of free fatty acids that were converted to ketone bodies in the liver. Moreover, degradation of squalene by ROS produced acetone in human skin and liver (Wisthaler & Weschler 2010). Mean squalene concentrations in subcutaneous fat, and skin, skeletal muscle, and liver in human tissues have been reported to reach 309.9, 148.4, 25.0, and 21.8 μg per gram of wet weight, respectively (Liu et al., 1976). Since squalene is a major constituent of human subcutaneous fat, skin, skeletal muscle, and liver (Liu et al., 1976), an increase in levels of acetone from human skin after handgrip exercise might be due to degradation of squalene in forearm skin, skeletal muscle, and liver. (Fruekilde et al., 1998; Wisthaler & Weschler 2010).

6-Methyl-5-hepten-2-one concentrations in skin gas also significantly increased after handgrip exercise compared with levels at rest. Since its formation is also induced by oxidative degradation of squalene (Wisthaler & Weschler 2010), an increase in levels of acetone in human skin after handgrip exercise might be due to degradation of squalene in forearm skin, skeletal muscle, and the liver (Fruekilde et al., 1998; Wisthaler & Weschler 2010).

On the other hand, there was no significant difference in acetaldehyde and hexanal concentrations between at rest and after handgrip exercise. Although the mechanism of acetaldehyde and hexanal formation is not yet clearly understood, bacteria in the gut are known to metabolize pyruvic acid to acetaldehyde via pyruvate decarboxylase dehydrogenase (Turner et al., 2008). Acetaldehyde and ethanol are known to form during yeast/glucose fermentation (Smith et al., 2003; Turner et al. 2008). Moreover, acetaldehyde is generated by oxidative degradation of linolenic acid (Turner et al., 2008; Frankel et al. 1980; Fujisaki et al. 2002) and during cell apoptosis in the liver (Smith et al., 2003; Clemens et al., 2002) and brain (Holownia et al., 1999). Therefore, the present study has established that the handgrip exercise did not affect oxidative degradation of linolenic acid and yeast/glucose fermentation.

In conclusion, the amount of acetone and 6-methyl-5-hepten-2-one released from forearm skin increased during dynamic handgrip exercise. Acetaldehyde and hexanal levels did not increase during exercise compared with levels at rest.

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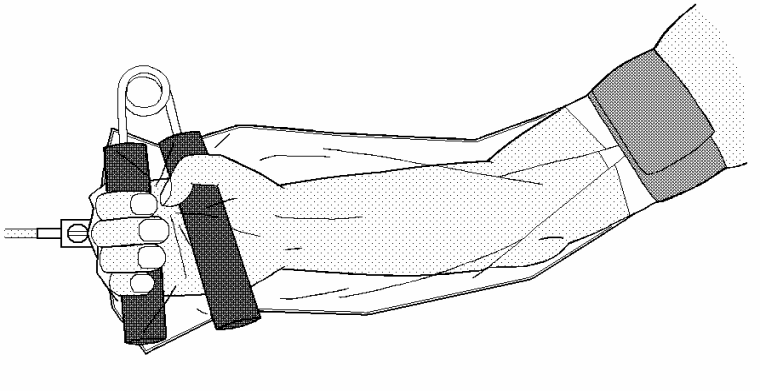
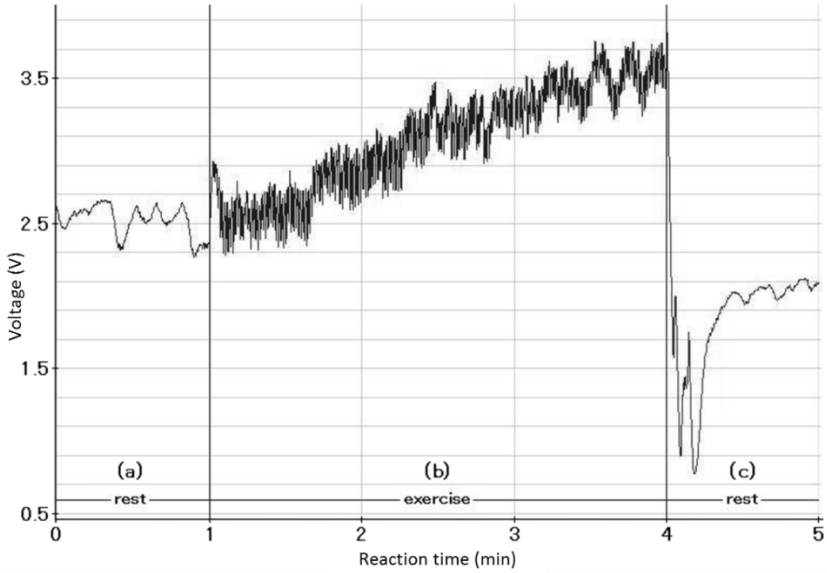
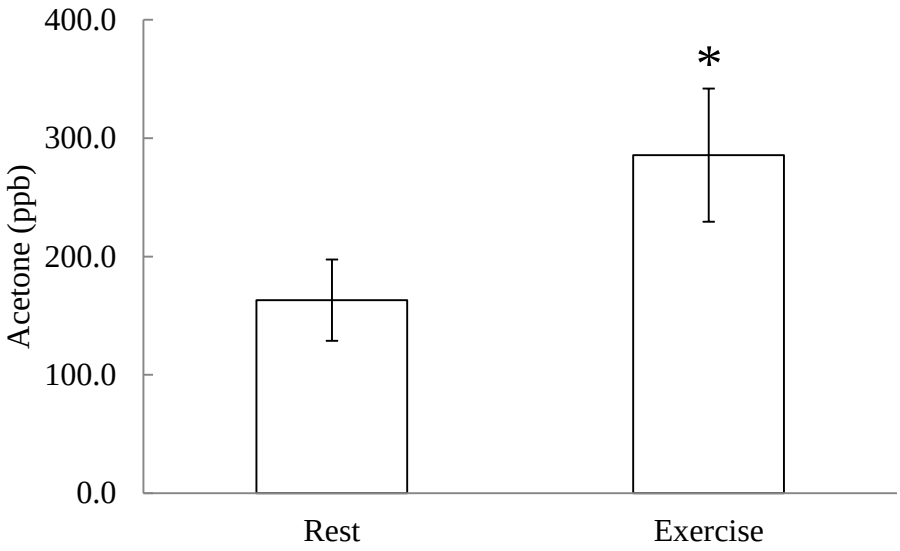


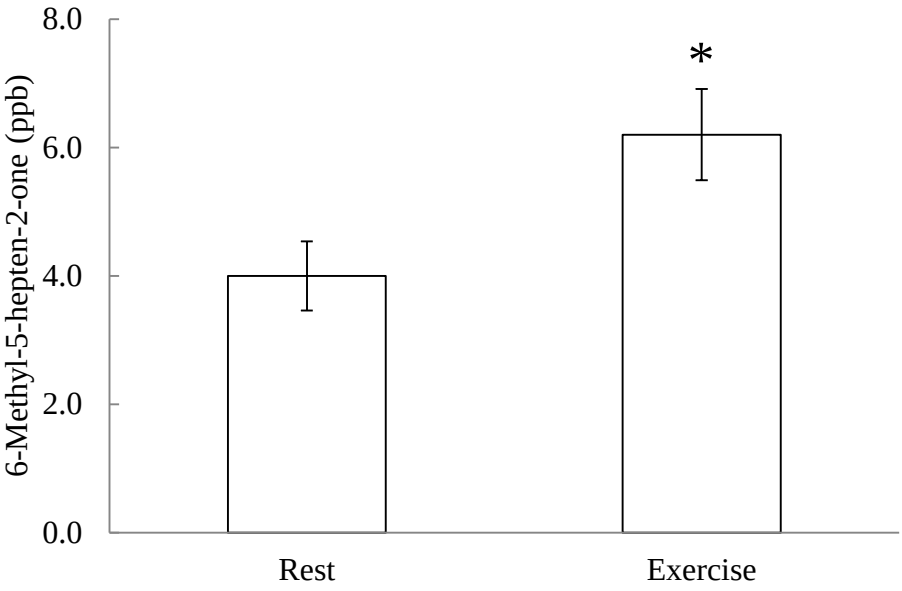
Graph Fig. 1: Sampling of skin gas during handgrip exercise.



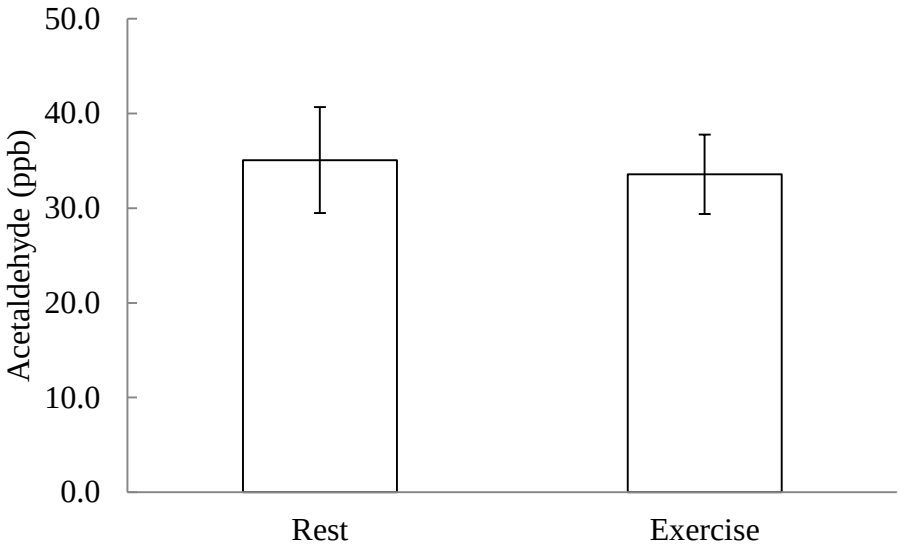
Graph Fig. 2: Chart of blood flow volume at rest (0–1 min) (a), during handgrip exercise (1–4 min) (b), and after exercise (4–5 min) (c).



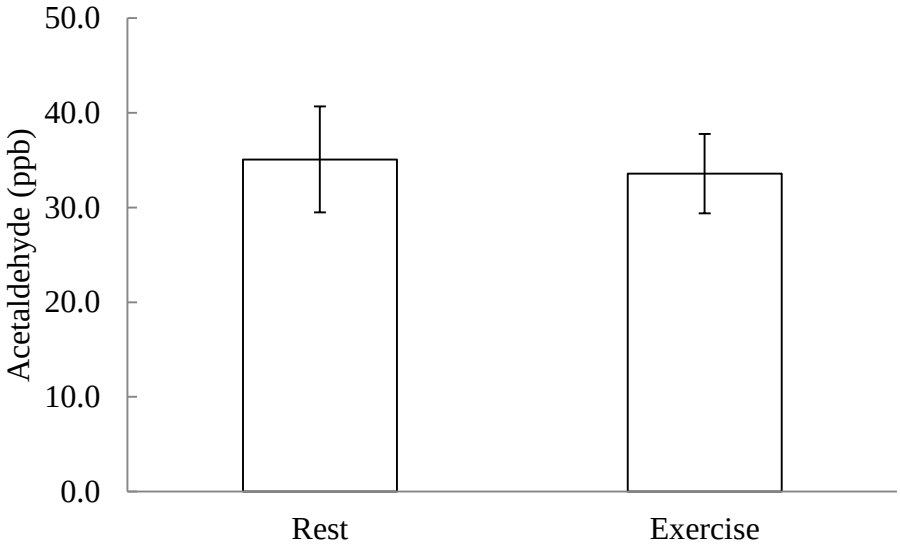
Graph Fig. 3: Concentration of acetone released from skin at rest and during handgrip exercise. Values are presented as mean ± SEM. *p < 0.05: significant difference from levels at rest.



Graph Fig. 4 Concentration of 6-methyl-5-hepten-2-one released from skin at rest and during handgrip exercise. Values are presented as mean ± SEM. *p < 0.05: significant difference from levels at rest.



Graph Fig. 5: Concentration of hexanal released from skin at rest and during handgrip exercise. Values are presented as mean ± SEM.



Graph Fig. 6: Concentration of hexanal released from skin at rest and during handgrip exercise. Values are presented as mean ± SEM