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Notes

Determination of Fatty Acids in Human Sweat during Fasting Using GC/MS

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Fatty acids (FAs) are biological molecules that are used as major metabolic fuels, and are concerned in important metabolic processes. We have performed a non-invasive and technically rapid and simple method for collecting sweat from humans, followed by GC/MS determination. The sweat was collected from each volunteer (the middle finger) by spraying 70% ethanol aqueous solution (no harmful solvent) into a 1.5-cm^3 plastic vial. Analysis of FAs in sweat showed that the sweat solution contains lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), oleic acid (C18:1), and stearic acid (C18:0). Here, it is demonstrated that FA concentrations for 4 young subjects correlated positively with percent of body fat ($r = 0.78$) and that the total FA levels for them increased progressively with increasing fasting time when a subject fasted throughout the experiment.

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Introduction

Fatty acids (FAs) are essential biological components in metabolism that are used as metabolic fuels, whereas elevated FA levels are commonly associated with obesity: a major risk factor for cardiovascular disease, hyperlipemia, and insulin resistance.[1,2] Concentrations of FAs in blood are elevated due to obesity, since triglycerides (TG) accumulated as excess energy in adipose tissue lead to release as the FAs.[1] Fast and starvation also cause an increase in liberation of FAs from TG adipose tissue, while blood glucose drops to a lower level,[1,3] leading to the increase of low-density lipoprotein (LDL) cholesterol levels.

In recent years, non-invasive analysis has attracted a great deal of attention because of major demands for health care. Advantages for sweat analysis of non-invasive methods include facilitated sample collection and no risk factor for infection; moreover, samples can be collected as many times as needed with much less stress. Various biological substances had already been found in human sweat,[4] such as some inorganic ions,[5-8] amino acids,[9,10] and lactic acids.[8,11,12] FAs, the important compounds in sweat, have also been observed with procedures by collection of sweat[12] and by ingestion of linseed oil.[13] However, there are no reports of disease-related analysis of sweat for FAs. In this study, we describe a rapid and simple method for collection of FAs for sweat analysis. The levels of FAs in sweat have a correlation with the body mass index (BMI, kilograms per square meter), and change in the amount of FAs in sweat during fasting.

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Experimental

Equipment

GC/MS analysis was performed on a QP-5050A (Shimadzu Corp., Kyoto, Japan). The GC separation of FAs was carried out on a DB-5MS capillary column (30 m in length, 0.25 mm in i.d., 0.25 μm in film thickness; Agilent J&W) coated with (5%-phenyl)-methylpolysiloxane.

The injection volume of the sample solution was 2 mm^3. The flow rate of helium flow as the carrier gas was regulated at 2.1 cm^3 min^{-1}. The injector and detector temperatures were 250 and 260°C, respectively. The oven temperature was held at 30°C for 1 min, then increased to 170°C at 30°C min^{-1} and to 180°C at 5°C min^{-1}, and finally to 220°C at 3°C min^{-1}. The analysis time was 21 min.

The MS detection was performed in a selected ion monitoring mode (SIM). In addition to the molecular ion M^+, the peak at 73 was commonly detected for all the FAs and the I.S. The mass spectrometer was operated in an electron impact ionization mode (EI) at 70 eV.

Chemicals

All the FAs (lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), oleic acid (C18:1) and stearic acid (C18:0)), and methyl decanoate (C10:0) used as the internal standard (I.S.), were of analytical grade, and were purchased from Wako (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Preparation of standard solutions

Six stock standard solutions containing the I.S. were separately prepared by dissolving fatty acid standards in a 70% ethanol–water solution (final concentrations 100 μM). Except for I.S. the mixed standard solutions were prepared by mixing

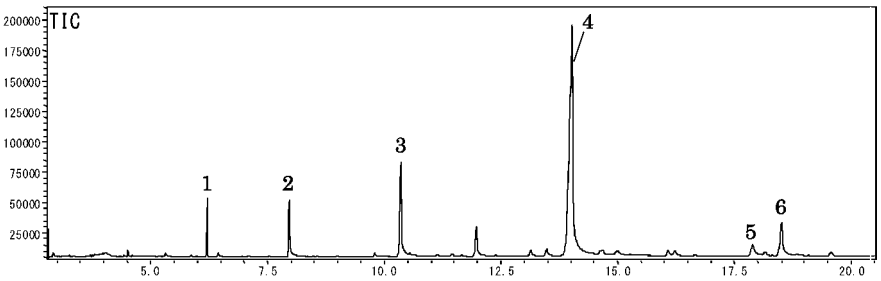

Fig. 1 Typical chromatogram of a sweat sample with the internal standard (I.S.). Peaks: 1, methyldecanoate (I.S.); 2, lauric acid; 3, myristic acid; 4, palmitic acid; 5, oleic acid; 6, stearic acid.

Table 1 Subject information and data of 5 FAs

Subject	Age	Gender	Body fat, %	Fatty acid/pmol cm^{-2} min^{-1}					Total fatty acid/ pmol cm^{-2} min^{-1}
				Lauric, C12:0	Myristic, C14:0	Palmitic, C16:0	Oleic, C18:1	Stearic, C18:0	
1	25	Female	19	6.2	11.4	32.2	1.0	1.1	52.0
2	22	Male	14	41.8	11.1	34.5	1.0	1.8	90.2
3	23	Male	18	31.1	22.7	91.6	2.8	6.5	154.7
4	23	Male	23	32.7	99.8	170.7	55.8	45.3	404.3

the 5 standard solutions in a small vial (micro-centrifuge tube of inner volume 1.5 cm^3, Sorenson, West Salt Lake).

After evaporating to dryness with a centrifugal evaporator at 85°C (EYELA, CVE-2000, Tokyo Rikakikai Co., Ltd., Tokyo, Japan), the residue was dissolved by adding 15 mm^3 of I.S. Then 2 mm^3 aliquot of the standard solution was injected into the GC/MS.

Preparation of sweat sample

After washing the middle finger with tap water for 10 s and wiping the finger with a tissue paper (Kimwipes-200, supplied by Kulesia, Tokyo) wetted with 70% an ethanol-water solution, the finger was rinsed with distilled water for 10 s. After 15 min, sweat on the palmar surface of the middle finger was collected in the small vial (1.5 cm^3) after spraying the solution by an inhaler (EW618, DC3V, Panasonic Electric Works Co., Ltd., Japan). The vial sample was dried in the centrifugal evaporator at 85°C, and 15 mm^3 of I.S. added as described above. Then 2 mm^3 of the sample solution was injected into a GC/MS.

Informed consent was obtained after the purpose and methods of the investigation had been fully explained. Sweat samples were collected from four healthy volunteers who were 3 males aged 22 – 23 years, weighing 53 – 75 kg and 1 female aged 25 years, weighing 47 kg. These four healthy subjects have not taken food for 12 h before the first sweat sampling for mat metabolism (test). Moreover, no food has been taken during experiment. Sweat samplings were successively performed from the palmar surface of the middle finger.

Results and Discussion

Linearity

The linearity of the relationship between GC peak area and FA concentrations was confirmed by analyzing standard samples. The calibration curves were obtained by dilution of the mixed standard samples to different concentrations. The relative peak area ratio for each FA obtained by GC was proportional to the logarithm of the corresponding FA concentration. The correlation coefficient was 0.98 (0.25 – 1.5 mM) for lauric acid, 0.99 (2.0 – 6.0 mM, n = 5) for myristic acid, 0.99 (3.0 – 7.0 mM, n = 5) for palmitic acid, 0.92 (1.5 – 5.5 mM, n = 5) for oleic acid, and 0.89 (2.0 – 6.0 mM, n = 5) for stearic acid, respectively.

Chromatographic analysis of sweat samples

Figure 1 shows the typical total ion chromatogram of the FAs in a sweat sample. The 6 peaks identified were assigned to I.S. (1), lauric acid (2), myristic acid (3), palmitic acid (4), oleic acid (5), and stearic acid (6), respectively, based on retention times (6.2, 8.0, 10.3, 14.0, 17.9, and 18.5 min). This indicates their presence in the human sweat. The retention times are in proportion to the chain length of the FAs. The FAs were detectable at levels of pmol cm^{-2} min^{-1} in the sweat sample of all the subjects. The FA contents in the sweat are approximately consistent with those in the superficial skin.[14]

Sweat FA levels

A sweat sample was collected from the subjects (3 males and 1 female) while fasting. The subject information and the obtained results are listed in Table 1. This indicate the presence of FAs in all subjects' sweats. Except for Subject 2, who has the highest concentration of palmitic acid,[12] palmitic acid was present at 25.3% of FAs on skin surface.[14] Subject 4 is obese with 23% body fat body and the total secretion rate of FA is correspondingly about 8 times higher than that of Subject 1.

It can be seen in the primary experiment that there was a positive correlation between the total FA secretion and the percent of body fat, with the correlation coefficient r = 0.78, particularly for palmitic acid (r = 0.81); moreover one can see that there was a weak correlation between the total rate of FA secretion and the BMI with r = 0.61.

Time-course of sweat FA levels

Sweat samples were taken successively every 1 h from Subject 1 (eight samples in total) under a fasting condition, and

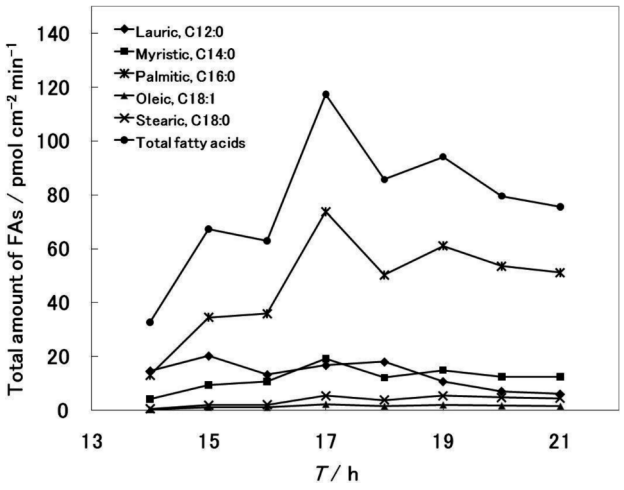

Fig. 2 Time-course of FAs rate levels in sweat.

collected into the vial by spraying 70% ethanol–water solution toward middle of finger with the inhaler. Figure 2 shows the time-course of FA secretion rate in sweat over 21 h of fasting. The concentrations of total FAs in sweat increased along with increasing time of fasting. In contrast, however, only lauric acid decreased approximately linearly with the fasting time. The result suggested that the FAs in sweat were released from TG of adipose tissue as an energy source into sweat through blood during longer fasting times. Thus, the FAs concentration in sweat is a promising indicator of TG levels in blood.

It has been reported that fasting time is related to increased FA levels in plasma.[1,3] Additionally, a good correlation was obtained between FA levels in sweat and in blood.[13] To our knowledge, however, the present work gives the first report on FAs in sweat which are elevated during fasting.

Conclusions

FAs in sweat are an important physiologic and metabolic marker, and possibly responsible for TG. We have shown that FAs are present in human sweat and particularly that palmitic acid is at the highest level in all the subjects, except one subject. The FA levels in sweat were correlated well with body mass index (r = 0.78). Moreover, we found that the FA concentrations increased gradually during the experimental fasting of a subject. This rapid and simple method for the sweat collection provides non-invasive determinations of FAs in sweat and possibility of application to diagnosis of serum TG level in adipose tissue.

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