Annals of Obesity & Disorders

Open Access

Austin Publishing Group

Special Article - Overweight

Changes in the 5'- AMP Concentration of Skeletal Muscles on Acetic Acid Treatment Under Fed or Starved Conditions in Rats

Araki A, Yoshimura Y, Maruta H, Kimoto M, Takahashi Y and Yamashita H*

Graduate School of Health and Welfare Science, Okayama Prefectural University, Japan

***Corresponding author:** Hiromi Yamashita, Graduate School of Health and Welfare Science, Okayama Prefectural University, Japan

Received: September 15, 2016; **Accepted:** October 12, 2016; **Published:** October 14, 2016

Abstract

Acetic acid is an endogenous compound produced and utilized for biological fuel under starved conditions. Under fed conditions, the acetic acid administered would be metabolized to acetyl-CoA with concomitant formation of intracellular 5'-Adenosine Monophosphate (AMP) via the catalytic activity of acetyl-CoA synthetase in the cytosol. Increase in intracellular AMP concentration would lead to the activation of 5'AMP-Activated Protein Kinase (AMPK). Acetic acid may have a potential role in regulating energy metabolism in muscles through the activation of AMPK. The objective of this study was to investigate the metabolic function of acetic acid. Under fed conditions, AMP concentration increased in muscles after administration of acetic acid. However, under starved conditions, the concentration of AMP remained unchanged. Phosphorylated AMPK also increased under fed conditions. The results indicate that orally administered acetic acid functions differently depending on fed and starved conditions, plays role in the activation of AMPK, and effects preventing obesity through the AMPK activation under fed conditions.

Keywords: AMP; AMPK; Acetyl-CoA; Acetic acid; Skeletal muscles

Abbreviations

AMP: 5'-Adenosine Monophosphate; AMPK: 5'AMP-Activated Protein Kinase; HPLC: High-performance Liquid Chromatography; BSA: Bovine Serum Albumin; SE: Standard Error; ANOVA: Analysis of Variance.

Introduction

Acetic acid is the main component of vinegar and is also found in ruminants as a product of bacterial fermentation [1,2].

Under starved conditions, acetic acid is utilized as a biological fuel. In contrast, under fed conditions, orally administered acetic acid is converted to acetyl-CoA with concomitant formation of intracellular AMP in the cytosol [2,3]. Increase in intracellular AMP concentration leads to the activation of AMPK, which acts as the master switch of energy metabolism [4-7] and stimulates fatty acid oxidation. Previously, we reported that orally administered acetic acid reduced pathological conditions in rats, through the reduction of lipogenesis and protection against fat accumulation. Furthermore, in animals, the acetic acid administered accelerates the phosphorylation of AMPK, expression of myoglobin and GLUT4 in skeletal muscles, and oxygen consumption rate [8,9]. Physiological role of administered acetic acid under different physiological conditions such as fed and starved has not yet been investigated. The purpose of this study was to investigate the metabolic function of administered acetic acid in skeletal muscles through monitoring changes in the AMP level under fed or starved conditions.

Materials and Methods

Experimental animals

Six-week-old male SD rats (n=5-11) were housed individually in an air-conditioned room at approximately 25°C with alternating 12h periods of light and dark.

For examining acetic acid metabolism, rats were administered 1% v/v acetic acid at 5 ml/kg body weight (52.5 mg/kg body weight), and they were anesthetized by isoflurane in 2-60 min after injection of acetic acid, under fed or starved (48h) conditions. Subsequently, the soleus and gastrocnemius muscles were obtained; they were freeze-clamped in liquid nitrogen and stored at -80°C.

The care and use of the animals in this study followed the guidelines of Okayama Prefectural University (No.27-3) and the laws and notifications of the Japanese government.

Nucleotides analysis

Lyophilized samples were homogenized with ice-cold 0.5N perchloric acid, neutralized with 5N potassium hydroxide and centrifuged. The concentrations of AMP, ADP, and ATP in the extracts of the skeletal muscle were determined by reverse-phase HPLC analysis.

Western blotting

Rat tissues were suspended in solution and homogenized. The homogenate was centrifuged. The proteins run on the gel were transferred onto a polyvinylidene difluoride membrane (Merch, DA, Germany). After the membrane was treated with 3% BSA, it was incubated with the primary anti-AMPK antibody, pThr172 AMPK from Cell Signaling Technology (MA, USA). The membrane was incubated with an HRP-conjugated secondary antibody. The chemiluminescent reaction was performed with ImmunoStar LD (Wako Pure Chemical Industries Ltd., Japan), and chemiluminescent signals were visualized and quantified with ImageQuant LAS-4000

Citation: Araki A, Yoshimura Y, Maruta H, Kimoto M, Takahashi Y and Yamashita H. Changes in the 5'- AMP Concentration of Skeletal Muscles on Acetic Acid Treatment Under Fed or Starved Conditions in Rats. Ann Obes Disord. 2016; 1(3): 1013.

Table 1: Adenosine nucleotides in muscles after oral administration of acetic acid under fed or starved conditions

Adenosine nucleotides (μmol/g of protein) in skeletal muscles of 7-week-old male SD rats orally administered 52.5mg/kg of BW acetic acid for the indicated times under soleus fed (a), soleus starved (b), gastrocnemius fed (c), and gastrocnemius starved (d) conditions. Each data value is expressed as the mean ± standard error (SE) (n=5~11). Significant differences among groups analyzed with one-way ANOVA followed by the Turkey- Kramer post hoc test. Groups without the same letter are significantly different (p<0.05).

a. Soleus muscle (fed condition).

Time (min)	ATP	ADP	AMP	Total
	μmol/g			
0	11.6±0.9	2.8±0.2	0.34±0.02a	14.7±1.0
2	11.8±0.8	2.6±0.4	0.36±0.03a	14.7±1.0
5	12.5±0.8	3.2±0.5	0.42±0.05ab	16.0±1.0
10	12.3±1.0	3.6±0.4	0.44±0.04ab	16.4±1.1
30	12.4±1.1	3.9±1.2	0.63±0.13b	16.9±0.5
60	11.4±0.9	2.7±0.5	0.59±0.08b	14.7±1.0

b. Soleus muscle (starved condition).

Time (min)	ATP	ADP	AMP	Total
	μmol/g			
0	16.0±0.5	2.3±0.2	0.16±0.02a	16.2±2.4
2	15.5±1.0	2.7±0.2	0.15±0.02a	16.9±2.1
5	16.5±0.7	2.8±0.3	0.13±0.01a	19.4±0.9
10	16.3±0.9	3.1±0.3	0.26±0.03b	19.6±1.4
30	15.7±2.2	2.8±0.4	0.21±0.02ab	18.7±2.6
60	16.2±1.7	2.7±0.2	0.16±0.02a	19.1±1.8

c. Gastrocnemius muscle (fed condition).

Time (min)	ATP	ADP	AMP	Total
	μmol/g			
0	11.5±0.9	3.3±0.2	0.62±0.08a	15.5±1.0
2	10.8±0.4	2.8±0.0	0.31±0.05b	14.0±0.3
5	11.9±0.5	2.7±0.1	0.22±0.00b	14.8±0.5
10	11.9±0.5	2.9±0.2	0.32±0.04b	15.1±0.7
30	11.2±0.4	3.0±0.2	0.33±0.05b	14.5±0.6
60	10.1±0.6	2.7±0.1	0.32±0.04b	13.1±0.6

d. Gastrocnemius muscle (starved condition).

Time (min)	ATP	ADP	AMP	Total
	μmol/g			
0	12.9±0.8	2.4±0.2	0.23±0.08	15.3±0.5
2	13.8±0.4	2.6±0.4	0.15±0.03	16.6±0.7
5	13.7±0.5	2.2±0.1	0.11±0.01	16.0±0.5
10	13.0±0.4	2.8±0.7	0.12±0.01	15.9±0.5
30	15.2±0.6	2.4±0.1	0.10±0.02	17.7±0.7
60	13.6±0.6	2.2±0.1	0.13±0.02	15.9±0.8

and Multi Gauge V3.2 analyzing software (Fujifilm, Tokyo, Japan).

Statistical analysis

Data are expressed as mean ± SE. Statistical differences between multiple groups were compared by one-way analysis of variance (ANOVA) followed by Tukey-Kramer post hoc analysis (Mulcell 2005). Differences between groups were considered statistically significant at $p < 0.05$.

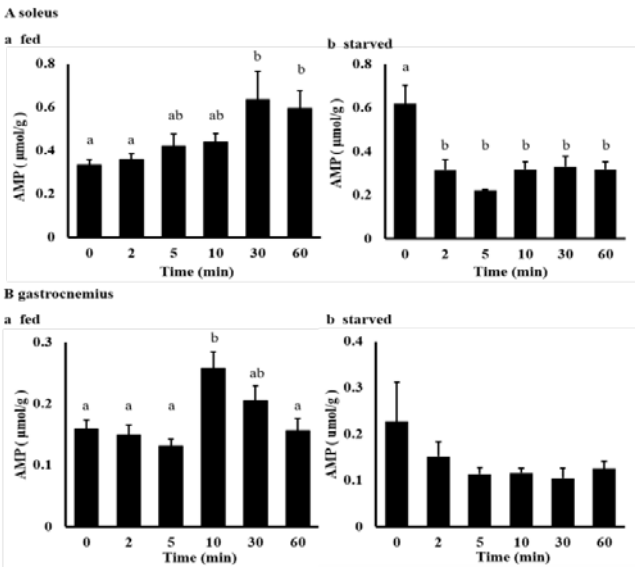

Figure 1: Change in AMP concentration in soleus (A) and gastrocnemius (B) muscles after oral administration of acetic acid under fed (a) or starved (b) conditions.

Acetic acid (52.5mg/kg of BW) was orally injected into 7-week-old male SD rats under fed or starved condition. Each data value is expressed as the mean ± SE for five to eleven rats. Significant differences among groups analyzed by Turkey- Kramer. Groups without the same letter are significantly different (p<0.05).

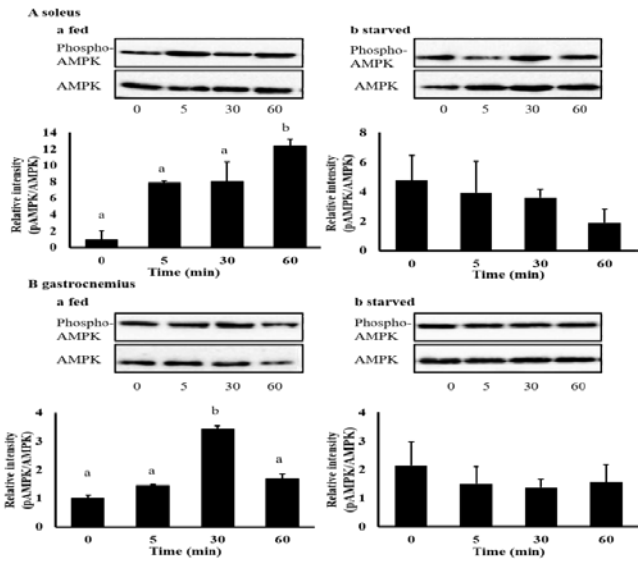

Figure 2: Effect of acetic acid administration on the phosphorylation of AMPK in soleus (A) and gastrocnemius (B) muscles after oral administration of acetic acid under fed (a) or starved (b) conditions.

Acetic acid (52.5mg/kg of BW) was orally injected into 7-week-old male SD rats (n=3) under fed or starved condition and phosphorylated AMPK was analyzed by western blotting. The relevant amount of the p-AMPK was normalized to the amount of AMPK. Each data value is expressed as the mean ± SE shown as relative intensity normalized to the value of 0min group. Significant differences among groups analyzed by Turkey- Kramer. Groups without the same letter are significantly different (p<0.05).

Results and Discussion

Effect of acetic acid on the change in AMP level in skeletal muscles

In our previous study, we reported that when acetic acid was orally administered to SD rats, it was readily taken up into the blood stream and was absorbed into tissues [8].

Under fed conditions, the acetic acid administered was converted to acetyl-CoA with concomitant formation of AMP. Under fed conditions, the AMP content of the skeletal muscles significantly increased in 10 to 60 min after injection of acetic acid (Table 1a,c, Figure 1Aa,Ba). In contrast, under starved conditions, the AMP content of the soleus muscle decreased in 2 min after the injection, and in the gastrocnemius muscle was not significantly changed. (Table 1b,d, Figure 1Ab,Bb). This finding indicates that AMP would accumulate in skeletal muscles on administration of acetic acid under fed conditions rather than starved conditions.

Phosphorylation of AMPK on treatment with acetic acid

Phosphorylation of AMPK significantly increased in 30-60 min after injection of acetic acid in soleus and gastrocnemius muscle under fed conditions (Figure 2Aa,Ba). AMPK acts as the key metabolic master switch and regulates a number of enzymes involved in lipid homeostasis. Activation of AMPK occurs by an increase in AMP. In our previous study, we found that administration of acetic acid under fed conditions resulted in suppression of lipid accumulation and lower weight gain for rats than water administered rats [8,9]. In this study, under fed conditions, administration of acetic acid led to the generation of AMP, which might be produced mainly in cytosol, and led to the activation of AMPK. While under starved conditions, it was found to lead much lesser accumulation of AMP and the level of phosphorylated AMPK remained unchanged. Even after administration of acetic acid (Figure 2Ab,Bb).

Conclusion

On the basis of these observations, we suggest that oral administration of acetic acid has a possible role in lipid metabolism in muscles and may also play a role in fighting obesity and obesity-linked type 2 diabetes through the activation of AMPK in fed condition.

Acknowledgment

This work was supported by the [JSPS KAKENHI] under Grant Number [15K07437] for Grant-in-Aid for Scientific Research (C) and the [JSPS KAKENHI] under Grant Number [26850087] for young scientist (B).

References

1. Ballard FJ. Supply and utilization of acetate in mammals. Am. J. Clin. Nutr. 1972; 25: 773-779.
2. Knowles SE, Jarrett IG, Filsell OH, Ballard FJ. Production and utilization of acetate in mammals,Biochem.J. 1974; 142: 401-411.
3. Zydowo MM, Smoleński RT, Swierczyński J. Acetate-induced changes of adenine nucleotide levels in rat liver. Metabolism. 1993; 42: 644-648.
4. Gowans GJ, Hawley SA, Ross FA, Hardie DG. AMP is a true physiological regulator of AMP-activated protein kinase by both allosteric activation and enhancing net phosphorylation. Cell Metab. 2013; 18: 556-566.
5. Moore F, Weekes J, Hardie DG. Evidence that AMP triggers phosphorylation as well as direct allosteric activation of rat liver AMP-activated protein kinase. A sensitive mechanism to protect the cell against ATP depletion. Eur J Biochem. 1991; 199: 691-697.
6. Winder WW, Hardie DG. AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. Am J Physiol. 1999; 277: 1-10.
7. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest. 2001; 108: 1167-1174.
8. Yamashita H, Fujisawa K, Ito E, Idei S, Kawaguchi N, Kimoto M. et al. Improvement of obesity and glucose tolerance by acetate in Type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. BiosciBiotechnolBiochem. 2007; 71: 1236-1243.
9. Yamashita H, Maruta H, Jozuka M, Kimura R, Iwabuchi H, Yamato M, et al. Effects of acetate on lipid metabolism in muscles and adipose tissues of type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. BiosciBiotechnolBiochem. 2009; 73: 570-576.

Citation: Araki A, Yoshimura Y, Maruta H, Kimoto M, Takahashi Y and Yamashita H. Changes in the 5'- AMP Concentration of Skeletal Muscles on Acetic Acid Treatment Under Fed or Starved Conditions in Rats. Ann Obes Disord. 2016; 1(3): 1013.