

Removal of ovarian hormones affects the ageing process of acetate metabolism

Yoshihisa Urita¹, Toshiyasu Watanabe¹, Tsunehiko Imai², Yasuyuki Miura³, Naohiro Washizawa³, Masaki Sanaka¹, Hitoshi Nakajima¹ and Motonobu Sugimoto¹

Departments of General Medicine and Emergency Care¹, Environmental and Occupational Health², and Surgery³
Toho University School of Medicine, Tokyo, Japan.

Background: Despite a close association between gastrointestinal motility and sex hormones, it has been unknown whether ovarian hormones affect absorption and metabolism of nutrients. The aim of this study is, therefore, to evaluate metabolism of acetate in rats with age and the influence of ovariectomy on its change. **Methods:** Fourteen female rats of the F344 strain were used, and ¹³C-acetate breath test was performed at 2, 7 and 13 months of age. Seven rats were ovariectomized at three weeks of age (ovariectomy group) and the remaining seven rats were studied as control group. After 24-hr fasting, rats are orally administrated 1ml of water containing sodium ¹³C-acetate (100mg/kg) and housed in an animal chamber. The expired air in the chamber is collected in a breath-sampling bag using a aspiration pump. The ¹³CO₂ concentration is measured using an infrared spectrometer for 120 min and expressed as delta per mil. **Results:** The breath ¹³CO₂ excretion increased with time and peaked 30 min in control rats. In ovariectomized rats, the peak time of ¹³CO₂ excretion was prolonged to 40 min at 7 and 13 months of age. Cmax was significantly higher at 2 months of age but lower at 4 months of age in ovariectomized rats than in control rats. Those of two groups became equal at 7 months of age. **Conclusions:** From the viewpoint of acetate metabolism, removal of ovarian hormones might make rats to be precocious ones and accelerate ageing. (Urita Y, Watanabe T, Imai T, Yasuyuki Miura Y, Washizawa N, Masaki Sanaka M, Nakajima H, Sugimoto M. Removal of ovarian hormones affects the ageing process of acetate metabolism. *North Am J Med Sci* 2009; 1: 58-62).

Key words Acetate oxidation - ¹³C-acetate breath test – ovariectomy – aging

Corresponding to: Yoshihisa Urita, MD, PhD, FACC. 1Department of General Medicine, Toho University School of Medicine, 6-11-1, Omori-Nishi, Ota-Ku, Tokyo 143-8541, Japan. Tel 81-3-762-4151, Fax 81-3-3765-6518. Email: foo@eb.mbn.or.jp

Introduction

Gastrointestinal function is well preserved with ageing regarding the digestion and absorption of macronutrients, whereas it has been reported that the ageing modifies metabolism [1]. For example, ovarian hormones play an important role on metabolism. They regulate body composition in female rats, and the removal of ovarian hormones by ovariectomy causes an increase in both body lipid and protein content [2]. The effects of ovarian hormones on body composition are thought to be derived primarily from changes in energy intake. Toth et al [3] also reported that estradiol and progesterone regulate the growth of fat-free mass by altering skeletal muscle protein synthesis. Hormones regulate skeletal muscle development, especially those that contribute to sex differences in body composition. Therefore, menopause is associated with an increase in body mass and body fat distribution [4], as well as increased daily food consumption and decreased motor activity [5].

Ovarian hormones also influence gastrointestinal function because estrogen receptors have been found in the gastric and small intestinal mucosa [6]. It has been reported that premenopausal women and ovarian hormone-treated postmenopausal women had slower gastric emptying of solid compared with men [7]. At the tissue level, sex hormones inhibit muscle contractility in a variety of sites, including

colon [8], lower esophageal sphincter [9], and gall bladder. Thus, effects of sex hormones on gastrointestinal motility have been reported by many investigators, there are few studies about influence of ovarian hormones on absorptive function in the small intestine. The aim of this study is to evaluate metabolism of acetate in rats progressing through their life stages and the influence of ovariectomy on their change.

Material & Methods

Animals and laboratory: A total of 14 F344/DuCrj rats aged four weeks were purchased from CLEA Japan Inc. (Tokyo, Japan) and seven animals were ovariectomized at the age of three weeks (ovariectomy group). Animals were housed in a quiet and temperature- and humidity-controlled room (22-24°C and 50-60%, respectively) individually and allowed 24-h access to a powdered diet (CE-7, CLEA Japan Inc., Tokyo, Japan) and tap water, which was provided fresh daily between 900 and 1000 h. Each animal was handled, 15 min /day, on two consecutive days prior to the experiment. The weight of the rats was recorded every day, and daily water and food intake was measured between 900 and 1000 h. ¹³C-acetate breath test was performed in each animal at 2, 4, 7, and 13 months of age. All procedures were approved by

the Institutional Animal Care and Use Committee of Toho University, Tokyo, Japan.

Breath test: After 24-hr fasting, ^{13}C -acetate breath test was performed using the system for monitoring the $^{13}\text{CO}_2$ levels in expired air from small animals reported by Uchida et al [10]. Briefly, the system composed a desiccator that was used as animal chambers, a pump and breath-collecting bags. Aspirating the expired air of rats caused fresh air to automatically flow into the desiccator to replace it through a hole in the side of the chamber (Fig.1). After the rats were placed in the chamber for 10 minutes, 1200 mL of expired air was collected into the sampling bag as a baseline. Next, rats were orally administrated 1 mL of water containing sodium ^{13}C -acetate (100mg/kg) and housed in an animal chamber. The expired air in the chamber is collected in a breath-sampling bag using a tube and aspiration pump at 10-min interval for 120 min. The $^{13}\text{CO}_2$ concentration is measured using an infrared spectrometer and expressed as delta per mil.



Fig.1. A picture of the system used for collecting expired air of rats.

Statistical analysis: Results are reported as means (SD) unless otherwise indicated. The maximum values of $^{13}\text{CO}_2$ excretion between two groups were compared at each age using Student's t test. Repeated measures ANOVA were used to examine between group differences in breath $^{13}\text{CO}_2$ excretion, since we had measurements for all rats at all nine time points. An interaction between age and ethanol feeding was examined by two-way ANOVA. When ANOVA indicated differences among the groups, Turkey's HSD (honestly significant difference) procedure was used to identify which group differences accounted for the significant p value. All analyses were done by the Statistical Package (JMP v 6.0 in Japanese edition).

Results

Longitudinal changes in acetate metabolism with ageing: The average values of the $^{13}\text{CO}_2$ excretion at each sampling point after administration of ^{13}C -acetate (100mg/kg) are shown in Fig. 2 (control rats) and Fig. 3 (ovariectomized rats) at 2, 4, 7, and 13 months of age. The breath $^{13}\text{CO}_2$ levels after administration of ^{13}C -acetate increased and peaked at

30 min at all different ages in control rats. For control rats, $^{13}\text{CO}_2$ excretion at 7 months of age was equal to that at 13 months. $^{13}\text{CO}_2$ excretion was markedly enhanced at 4 months and plateau after that. This indicated that acetate metabolism would be activated at an early age and steady. The values in each time point were significantly lower at 2 months of age than at 4, 7, and 13 months of age ($p < 0.01$, Repeated measures ANOVA). The maximum value of mean $^{13}\text{CO}_2$ excretion is 933 ± 138 per mil at 2 months of age, 1506 ± 143 per mil at 8 months of age, and 1561 ± 271 per mil at 13 months of age. The maximum value of $^{13}\text{CO}_2$ excretion was significantly lower at 2 months of age than at 7 and 13 months of age ($p < 0.01$, paired t test), whereas there was no significant difference in $^{13}\text{CO}_2$ excretion among 4, 7 and, 13 months of age.

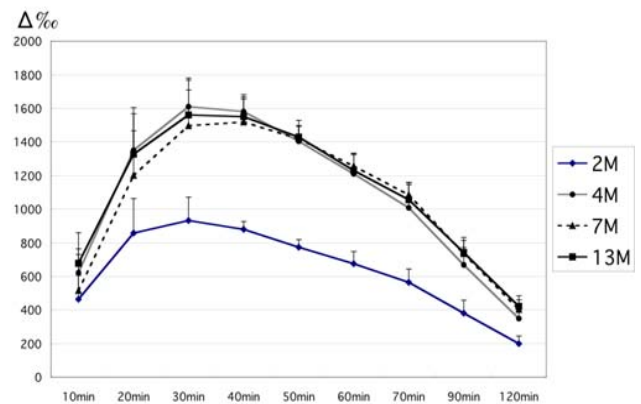


Fig. 2. $^{13}\text{CO}_2$ excretion after ingestion of ^{13}C -acetate in control rats.

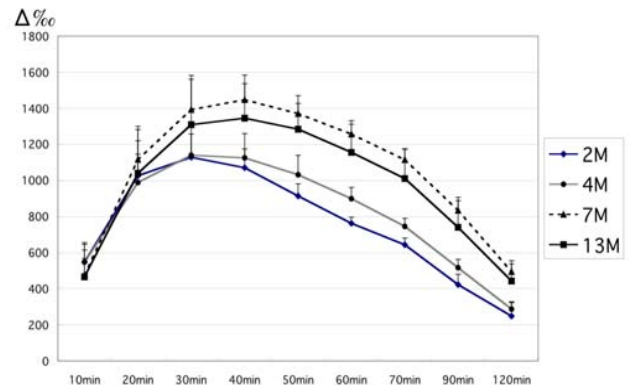


Fig. 3. $^{13}\text{CO}_2$ excretion after ingestion of ^{13}C -acetate in ovariectomized rats.

In ovariectomized rats, the breath $^{13}\text{CO}_2$ level also increased and peaked at 30 min at 2 and 4 months of age, whereas the peak time was prolonged to be 40 min at 7 and 13 months of age. The maximum value of mean $^{13}\text{CO}_2$ excretion is 1129 ± 130 per mil at 2 months of age, 1140 ± 158 per mil at 4 months of age, 1448 ± 191 per mil at 7 months of age, and 1346 ± 137 per mil at 13 months of age. The peak value of $^{13}\text{CO}_2$ excreted was highest at 7 months of age, followed by 13 months, 4 months and 2 months.

Compared to 2 months, $^{13}\text{CO}_2$ excretion was significantly greater at 4, 7, and 13 months of age. There was a significant interaction between age and $^{13}\text{CO}_2$ excretion (tests with contrasts in ANOVA; for F values, $df=8, 3, p<0.001$).

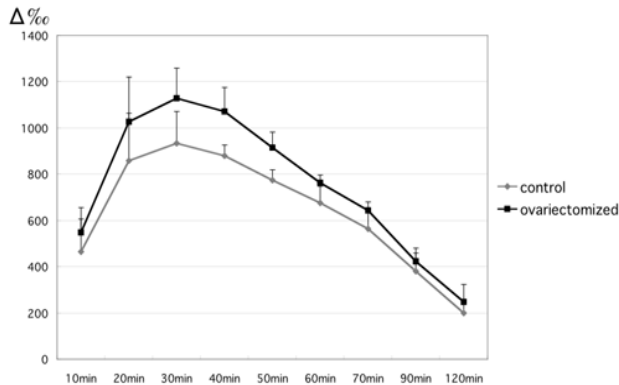


Fig. 4. Comparison of $^{13}\text{CO}_2$ excretion at 2 months of age between ovariectomized rats and controls.

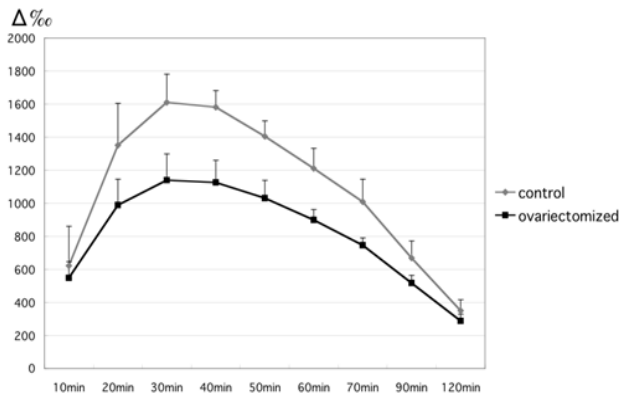


Fig. 5. Comparison of $^{13}\text{CO}_2$ excretion at 4 months of age between ovariectomized rats and controls.

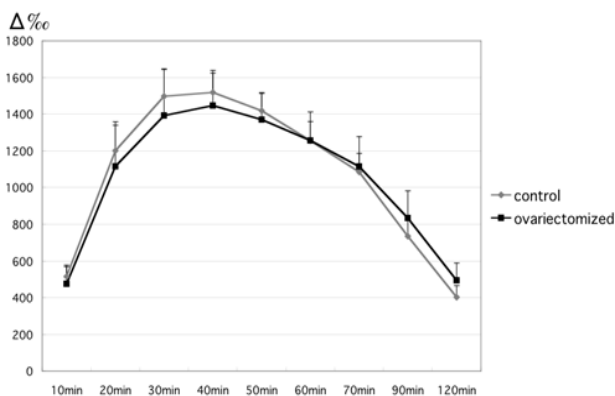


Fig. 6. Comparison of $^{13}\text{CO}_2$ excretion at 7 months of age between ovariectomized rats and controls.

Comparison of acetate oxidation between ovariectomized rats and controls: $^{13}\text{CO}_2$ levels in the expired air were excreted significantly more in ovariectomized rats at 2 and 4 months of age (tests with contrasts in ANOVA; for F values, $df=8, 3, p<0.01$), as shown in Fig. 4 and Fig. 5. At 7 months of age, $^{13}\text{CO}_2$ excretion in ovariectomized rats

was increased up to the approximately equal level to that in control rats (Fig. 6). At 13 months of age, ovariectomized rats had a significantly lower $^{13}\text{CO}_2$ excretion than control rats (tests with contrasts in ANOVA; for F values, $df=8, 3, p<0.01$) (Fig. 7).

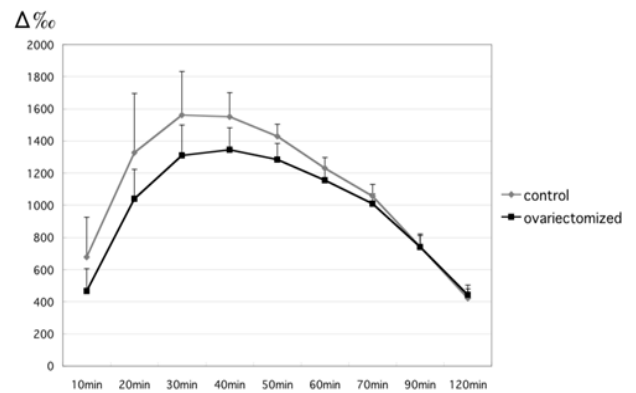


Fig. 7. Comparison of $^{13}\text{CO}_2$ excretion at 13 months of age between ovariectomized rats and controls.

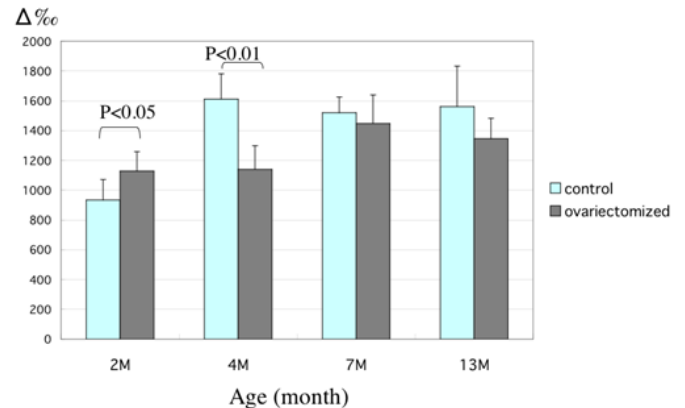


Fig. 8. Peak values of $^{13}\text{CO}_2$ excretion in each age.

The maximum value of $^{13}\text{CO}_2$ excretion is significantly higher at 2 months of age but lower at 4 months of age in ovariectomized rats than in control rats (Fig. 8). Those of two groups became equal at 7 months of age and the difference was getting greater after that. There were significant differences in the maximum value of $^{13}\text{CO}_2$ excretion between two groups at 2 months ($p<0.05$) and 4 months ($p<0.001$) of age.

Discussion

There have been few investigations of factors that regulate the concentration of acetate in the blood. Acetate concentration in the blood reflects the balance between rates of production and utilization. Since acetate is the major products of bacterial fermentation of the carbohydrate that enters the colon [11], the rate of fermentation is influenced by the rate of entry of carbohydrates into the colon. Under conditions of increased fat oxidation such as prolonged starvation and diabetes, endogenous acetate production predominates [12] although colonic fermentation is the

major source of blood acetate. Elevated acetate values in diabetics are attributed to an impaired acetate metabolism and to an increased availability of acetyl-CoA from various sources such as glucose and fatty acids [13]. A key enzyme (acetyl-CoA synthetase) of acetate metabolism is regulated by insulin [14]. In addition, the endogenous source from glucose and fatty acid metabolism is also likely to be important in mammals [15], and the elevated acetate levels in diabetics depend mainly on endogenous production or decreased metabolism rather than on colonic fermentation [16]. Thus, the serum acetate level is influenced by both endogenous and exogenous sources although it is derived primarily from colonic fermentation.

Acetate is rapidly absorbed during perfusion of the human jejunum and it has been reported that saturation of the absorption process occurred at concentrations greater than 40 mM [17]. In rats, it has been shown that hepatic acetate uptake is directly proportional to the concentration of acetate in the portal vein [13] and hepatic uptake of acetate averages >60% of that absorbed from the gut. Importantly, rat liver puts out acetate into the blood when the portal concentration falls below a minimum value of less than 0.2 mM, which is close to the Michaelis constant (K_m) for acetate of acetyl-CoA synthetase [13]. Like human diabetics, diabetic rats have elevated blood acetate concentrations because of increased production and release of acetate by the liver [18]. In the postabsorptive state, acetate is largely oxidized probably in the liver and contributed to almost 7% of total energy expenditure in healthy humans [19]. Unlike ruminants, glucose carbon is preferred substrate which is used more rapidly than acetate carbon in rats [20]. Acetate is assumed to be immediately converted into acetyl-CoA, which will then enter the TCA cycle. Exogenous acetate administered orally passes in part through the splanchnic bed and reaches the peripheral bloodstream, mixing with the endogenous circulating acetate [21].

Using a stable isotope, ^{13}C , the rate of exogenous acetate oxidation in the whole body could be chronologically investigated by monitoring the expiration of $^{13}\text{CO}_2$ in the respiratory gas. ^{13}C -acetate breath test has recently been used to assess gastric emptying [22]. The appearance of $^{13}\text{CO}_2$ in breath indicates that the substrate has been oxidized and hence absorbed through the gut [23]. The $^{13}\text{CO}_2$ excretion curve depends on mainly gastric emptying using liquid and solid meals with a high calorie. Using a water solution of ^{13}C -substrate without calories, the influence of gastric emptying on $^{13}\text{CO}_2$ excretion curves is dramatically reduced. The $^{13}\text{CO}_2$ excretion curve after an oral administration of ^{13}C -acetate solution reflects mainly both absorption and oxidation of substrate. In the present study, $^{13}\text{CO}_2$ excretion was markedly enhanced at 4 months and plateau after that in control rats, indicating that acetate metabolism would be activated at an early age and steady. Although gastrointestinal function is well preserved with ageing regarding the digestion and absorption of macronutrients, the ageing gastrointestinal tract becomes less efficient in absorbing vitamin B_{12} , vitamin D, and calcium [1]. Although it has been little known whether absorptive and oxidative function of acetate is preserved

with ageing, based on the results of the present study, its function was well preserved until 13 months of age in rats.

On the other hand, the peak time of breath $^{13}\text{CO}_2$ excretion was prolonged to be 40 min at 7 and 13 months of age in ovariectomized rats. The maximum value of $^{13}\text{CO}_2$ excretion is significantly higher at 2 months of age but lower at 4 months of age in ovariectomized rats than in control rats as shown in Fig. 8, suggesting that, regarding absorptive and oxidative function of acetate, ovariectomy may accelerate ageing process. It has been reported that ovariectomy that induced bone loss in the rat and postmenopausal bone loss share many similar characteristics including decreased intestinal absorption of calcium [24]. Ku et al. [25] has reported severe quantitative changes of argyrophil endocrine cells that are responsible for the production of gut hormones by ovariectomy. It is possible that these changes regulate gut motility and digestion including absorption. Osteoporetic experimental animals shows impairment of absorption of calcium ion and increase of absorption of cholesterol and other lipids [26], possibly resulting in an increase in both lipid and protein content.

The use of ^{13}C -acetate in studying plasma acetate oxidation has been questioned because the appearance of $^{13}\text{CO}_2$ is very low at rest [27]. It has been also reported that part of the substrate will be lost in the bicarbonate pool, or in non-oxidative pathways, and will accumulate in products of the TCA cycle [28]. Since acetate is immediately converted into acetyl-CoA, which will then enter the TCA cycle, the recovery of exogenous ^{13}C -acetate as $^{13}\text{CO}_2$ in breath can be used to calculate the rate of oxidation. Despite that diabetic patients have elevated plasma acetate concentrations; ^{13}C -acetate recovery has been reported to be lower [29]. It could be suggested that an increased rate of gluconeogenesis in the diabetic state would lead to lower acetate recovery. Similarly, impaired ^{13}C -acetate recovery found in ovariectomized rats is considered to associate with changes in glucose metabolism because intra-abdominal fat accumulation induced by ovariectomy is associated with glucose intolerance [30].

This is the first study to clarify changes in acetate metabolism with advancing age in a longitudinal study using same experimental animals. Breath tests are safely and noninvasively performed and can be repeated many times if necessary. However, a longitudinal human study to evaluate the association between acetate metabolism and ageing is time-consuming, and it is difficult to collect breath samples of small animals. The new system used for monitoring the $^{13}\text{CO}_2$ levels in expired air from small animals can resolve these problems. Furthermore, it developed that acetate metabolism including absorption and oxidation and its alteration with advancing age were changed remarkably by ovariectomy. Interestingly, ovariectomy may accelerate both maturity and ageing regarding acetate metabolism.

In conclusion, effect of ageing on acetate metabolism or oxidation is changed remarkably by ovariectomy. $^{13}\text{CO}_2$ excretion is significantly higher in ovariectomized rats at 2 months of age in each time point, suggesting that ovariectomy might enhance acetate metabolism at an early age. From the viewpoint of acetate metabolism, removal of

ovarian hormones might make rats to be precocious ones and accelerate ageing.

Conflict of Interest Disclosure: We hereby declare that there are not any potential conflicts of interest that are relevant to the manuscript.

Authors' contributions: Toshiyasu Watanabe, Tsunehiko Imai, and Yoshihisa Urita wrote the paper and contributed to acquiring data. Yasuyuki Miura and Naohiro Washizawa contributed to analyzing data. Masaki Sanaka and Hitoshi Nakajima contributed to drafting the manuscript. Motonobu Sugimoto contributed to enhancing its intellectual content.

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