Nutrition 29 (2013) 796–801

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Basic nutritional investigation

Pantothenic acid refeeding diminishes the liver, perinephrical fats, and plasma fats accumulated by pantothenic acid deficiency and/or ethanol consumption

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A R T I C L E I N F O

Article history:
Received 1 August 2012
Accepted 10 January 2013

Keywords:
Pantothenic acid
Ethanol
Deficiency
Fat
Rat

A B S T R A C T

Objective: Pantothenic acid (PaA) is a vitamin that is an integral part of coenzyme A (CoA). CoA is an essential coenzyme in fat metabolism. The aim of this study was to determine whether PaA deficiency causes the accumulation of tissue fats and, if so, can refeeding of PaA decrease such accumulated fat.

Methods: Weaning rats were fed the PaA-free diet for 30 d. Rats were then divided into two groups. One group was continuously fed the PaA-free diet, and the other was fed the PaA-containing diet for an additional 13 d. At the end of the experiment, liver fat and perinephric fat were weighed, and plasma triglyceride levels measured. An additional similar experiment was conducted in which rats consumed 15% ethanol instead of water.

Results: Fat that accumulated by consuming the PaA-free diet for 30 d was decreased by consuming the PaA-containing diet for an additional 13 d. Ethanol feeding elicited much greater accumulation of liver, perinephric, and plasma fats if rats were fed the PaA-free diet. In such cases, administration of PaA could decrease the accumulated fat.

Conclusion: PaA deficiency causes fat accumulation, and readministration of PaA decreases the tissue fat in rats fed the pantothenic acid–free diet. Ethanol accelerated the accumulation of fat in rats fed the PaA-free diet. PaA could be beneficial for decreasing accumulated tissue fat.

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Introduction

Pantothenic acid (PaA) as part of coenzyme A (CoA) is important for the formation of acetyl coenzyme A (acetyl CoA), which is the precursor for the biosynthesis and a key substrate of the catabolism of fatty acids. Compared with control animals, PaA deficiency can cause retarded growth [1], reduced adrenal function [2], severe anatomical and functional impairments of the testes with resulting loss of fertility [3], high serum levels of triglyceride (TG) and free fatty acids [4], and other effects [5]. Early nutritional research suggested that PaA deficiency produced non-specific deficiency diseases. Recently, PaA deficiency has been shown to reduce several parameters of sperm motility and to lower testicular weight [6] compared with control animals. PaA depletion from the culture medium has been demonstrated to suppress the proliferation and promote the differentiation of keratinocytes [7]. These recent studies suggest that PaA is an essential factor of sperm mobility and skin maintenance.

We previously found that ethanol consumption in rats fed a diet low in vitamins (including PaA) caused a decrease in PaA contents in the liver, blood, and urine [8]. We also reported [9] that administration of a large amount of fat to rats fed a diet containing sufficient amounts of vitamins decreased PaA contents in the liver, plasma, and urine. These findings [4,8,9] imagined that PaA deficiency and ethanol administration cause fat to accumulate in the body.

Thus, the objective of the present study was to ascertain whether PaA deficiency and/or ethanol consumption causes

This study was part of the project Studies on the Dietary Reference Intakes for Japanese (principal investigator, KS), which was supported by a Research Grant for Comprehensive Research on Cardiovascular and Lifestyle-Related Diseases from the Ministry of Health, Labour and Welfare of Japan.

KS carried out the design and drafted the manuscript. TF participated in its design and drafted the manuscript. SH and CS carried out the experiments. IA and MO helped in the design of the study. All authors approved the final manuscript.

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http://dx.doi.org/10.1016/j.nut.2013.01.002

disturbances in fat metabolism and an accumulation of body fats such as liver, perinephrical, and plasma fats, if so, whether refeeding of PaA can decrease the amount of accumulated fat.

Materials and methods

Chemicals

Vitamin-free milk casein, sucrose and L-methionine were purchased from Wako Pure Chemical Industries (Osaka, Japan). Corn oil was obtained from Ajinomoto (Tokyo, Japan). Gelatinized cornstarch, a mineral mixture (AIN-93G mineral mixture) [10] and a vitamin mixture (nicotinic acid-free AIN-93 vitamin mixture containing 25% choline bitartrate) [10] were from Oriental Yeast Co., Ltd. (Tokyo, Japan).

Calcium pantothenate ($C_{18}H_{32}N_2O_{10}$-Ca = 476.54) was purchased from Wako Pure Chemical Industries. All other chemicals used were of the highest purity available from commercial sources.

Animals and treatment

The care and treatment of experimental animals conformed to guidelines for the ethical treatment of laboratory animals set by the University of Shiga Prefecture (Shiga, Japan). Room temperature was maintained at ≈22°C and ≈60% humidity. A 12-h light–dark cycle was also in operation (06:00–18:00/18:00–06:00).

Experiment 1

Three wk old male Wistar rats obtained from CLEA Japan (Tokyo, Japan) were housed individually in metabolism cages (CT-10; CLEA Japan). They were then divided into two groups at 09:00, which was designated as the "zero time" of the experiment elapsed time (EET). One group (n = 5) was fed a PaA-containing diet and allowed to drink tap water ad libitum for 30 d; this was designated the positive control diet plus water (PCW) group (Table 1). The other group (n = 5) was fed the same diet (Table 1), but allowed to drink 15% ethanol (v/v) (consists of 15 mL of pure ethanol and 85 mL of tap water) ad libitum for 30 d; this was designated the positive control diet plus ethanol (PCE) group. Rats were then sacrificed. Livers were removed and the amount of liver fat and perinephric fat measured. Blood was also collected from the carotid artery and plasma obtained.

Experiment 2

Three wk old male Wistar rats obtained from CLEA Japan were housed individually in metabolism cages (CT-10). They were divided into two groups at 09:00, which was designated as the zero time of the EET. One group (n = 5) was fed the PaA-free diet and drank tap water ad libitum for 30 d; this group was designated the negative control diet plus water (NCW) group (Table 1). The other group (n = 5) was fed the same diet (Table 1), but drank 15% ethanol ad libitum for 30 d; this group was designated the negative control diet plus ethanol (NCE) group. Rats were then sacrificed. Livers were removed and the amount of liver fat and perinephric fat measured. Blood was also collected from the carotid artery and plasma obtained.

Experiment 3

Three wk old male Wistar rats obtained from CLEA Japan were housed individually in metabolism cages (CT-10). They were initially divided into two groups: NCW group and the NCE group at 09:00, which was designated as the zero time of the EET. When rats were freely fed the diet in experiment 2, the food intake was higher in the NCW group than in the NCE group. Therefore, food intake during the experiment was pair-fed in the two groups. Five rats from each group were sacrificed at 09:00 on day 31. Livers were removed and the amount of liver fat and perinephric fat measured. Blood also was collected from the carotid artery and plasma obtained.

The remaining rats (the number of rats in the NCW and NCE groups was 10) were then subdivided into two groups at 09:00 on day 31 and remained in their cages until 09:00 on day 44. Therefore, there were four groups from 09:00 on day 31 to 09:00 on day 44: NCW→NCW (n = 5), NCW→PCW (n = 5), NCE→NCE (n = 5), and NCE→PCE (n = 5). During the experiment, food intake among the four groups was pair-fed with the amount of NCE. Rats were sacrificed at 09:00 on day 44, and blood taken from the carotid artery. The livers and testes were removed and weighed. Perinephrical fat was removed and measured. Triglycerides (TGs) and the free form of PaA in plasma as well as the fat content and total content of PaA in the liver were measured.

Twenty four-hour urine samples (09:00–09:00) were collected in amber bottles containing 1 mL of 1 mol/L HCl and stored at −25°C until needed. The content of free PaA was measured.

Table 1
Compositions of the diets

	PaA-containing diet (g/kg diet) PC diet*	PaA-free diet (g/kg diet) NC diet
Casein	200	200
L-Methionine	2	2
Gelatinized cornstarch	469	469
Sucrose	234	234
Corn oil	50	50
Mineral mixture (AIN-93G-MX) [10]	35	35
Vitamin mixture (AIN-93-VX) [10]	10	0
PaA-free vitamin mixture (AIN-93-VX) [10]	0	10

PaA, pantothenic acid; PC, positive control; NC, negative control
The energy of the diet per g is 3.783 kcal
* PC diet contains 16 mg of calcium pantothenate per kg of diet, which corresponds to 14.7 mg of PaA.

Measurement of liver fat

The total content of fat in livers was measured using the method of Folch et al. [11].

Measurement of perinephric fat

Perinephric fat tissues were removed with scissors from abdominal operated rats, and weighed.

Measurement of plasma triglyceride

Plasma TG was measured with FUJI DRI-CHEM (Fujifilm Ltd., Tokyo, Japan).

Total PaA and free form of PaA

Frozen liver samples, ≈0.2 g, were thawed, minced, and added to 10 volumes of 50 mmol/L KH_2PO_4-K_2HPO_4 buffer (pH 7.0). The suspension was homogenized with a Teflon/glass homogenizer. The homogenate was incubated at 37°C overnight to convert the free form of PaA from the bound type of pantothenate compounds such as pantetheines, CoA, dephospho-CoA, and acyl-CoA. The reaction was stopped by putting the mixture into a boiling water bath for 5 min. After cooling, the mixture was centrifuged at 10 000g for 10 min at 4°C. The supernatant was retained and the precipitated materials were extracted again with 2 mL of water, and the supernatant retained. Both retained supernatants were combined, and the extract used for measuring PaA employing *Lactobacillus plantarum* ATCC 8014 [12].

The content of the free form of PaA in urine and serum was measured directly without incubating homogenates at 37°C overnight by using *Lactobacillus plantarum* ATCC 8014 [12].

Statistical analyses

The values in Tables and Figures are expressed as mean ± SEM. Student's t test was performed for comparison between PCW and PCE (Table 2), NCW and NCE (Figs. 1A, 2A, 3A, and Table 3). The main effects of diet (the PaA-free [NC] versus PaA-containing diets [PC]) and drink (ethanol administration [E] versus water administration [W]) during 13 d in experiment 3 were analyzed using a two-way analysis of variance (ANOVA), followed by Bonferroni's post hoc test. When two-way ANOVA indicated the presence of a diet–drink interaction, one-way ANOVA was conducted followed by Tukey's multiple-comparison test among the groups (Table 4 and Figs. 1B, 2B, 3B). Differences of $P < 0.05$ were considered to be statistically significant. Graph Pad Prism version 5.0 (Graph Pad Software, San Diego, CA, USA) was used for all analyses.

Results

Effect of ethanol consumption on fat and PaA contents in the liver in rats fed PaA-containing diet (experiment 1)

Weaning male rats were fed the PaA-containing diet (PC diet) and allowed to drink water or 15% ethanol for 30 days. Rats had free access to the food, water, or 15% ethanol during the experiment. Food intake was lower in the PCE group than in the PCW group. However, the energy intake (EI) between the two groups was almost identical because the rats in the PCE group were

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