

Effective dose of Alaska Pollack protein for increase of muscle

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Value are means ± SEM *: p<0.05, **: p<0.01 t-test

[Objectives] We have confirmed that the administration of Alaska pollack protein (APP) diet, which was **totally substituted APP for casein (Cas) in the AIN-93 diet significantly increased rat gastrocnemius muscle (GA) in 3 days after administration**, compared to Cas-based control diet. But the administration of amino acid mixture, which of the equivalent amino acid composition to APP(A-APP), was not effective in increasing skeletal muscle. It suggested that a protein or a degraded peptide of APP might be involved in the **efficacy by administration of APP, but not the amino acids**. In further study, we plan to investigate the effect of the administration of APP in the clinical trial. For **estimation of the dosage for humans**, we investigated the effective dose of APP for the skeletal muscle increasing effect of rat.

[Previous studies] An administration of APP increased rat skeletal muscle(Fig.1). Some report suggested that an administration of branched chain amino acids (BCAA) increased the skeletal muscle. However, **administration of A-APP is not effect** (Fig.2) and **BCAA of APP is lower than Cas** (Table 1). Therefore, these data suggested that the effect of APP was independent of BCAA.

Fig. 1 APP for Cas in the AIN-93 diet significantly increased GA in 3 days and 7days after administrations (Each group n = 8)

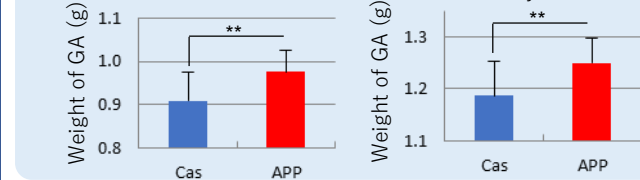


Fig. 2 APP or A-APP for Cas in the AIN-93 diet significantly increased rat GA in 3 days after administration (Each group n = 6)

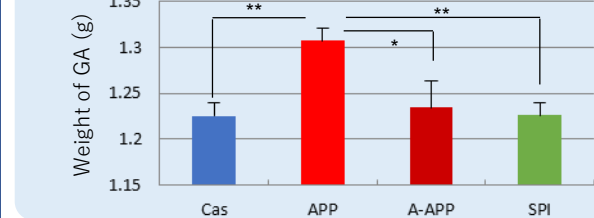
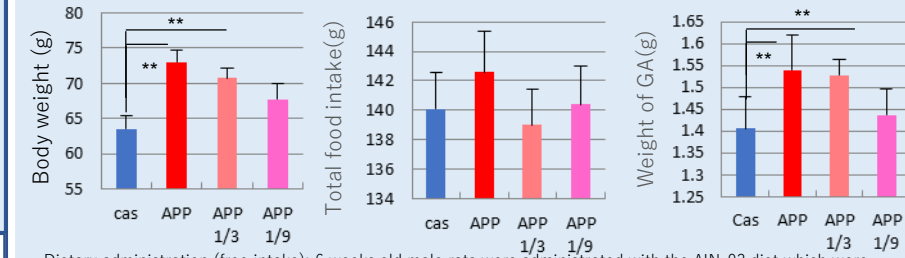


Table 1 Amino acid(AA) composition of APP and Cas

AA	Cas	APP	AA	Cas	APP	AA	Cas	APP	AA	Cas	APP
Asp	6.41	9.30	Ala	2.76	5.40	Leu	8.39	7.26	Arg	3.30	5.86
Thr	3.86	4.08	Val	5.77	4.40	Tyr	5.02	3.26	Trp	1.12	1.01
Ser	5.21	4.00	Cys	0.32	1.03	Phe	4.57	3.47	Pro	9.64	2.94
Glu	20.00	14.00	Met	2.52	2.66	Lys	7.14	8.41			
Gly	1.70	4.14	Ils	4.53	4.01	His	2.69	2.21			

[Exp. 1] APP for one third of Cas in the AIN-93 significantly increased GA.

Fig. 3 Administration with the AIN-93 diet which were substituted APP(Each group n = 10)

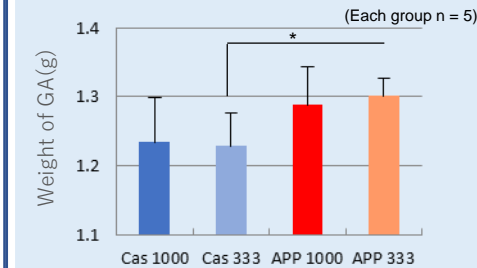


Dietary administration (free intake): 6 weeks old male rats were administered with the AIN-93 diet which were substituted APP for all, one third or one ninth of casein(APP 1/3, APP 1/9), or Cas-based control diet for 3 days. After administration, GA of rat were evaluated.

In the **APP all and one third administration group**, mass of the GA muscle and Body weight increased significantly, and in the APP one ninth administration group was no significant difference. All group was no significant difference of food intake.

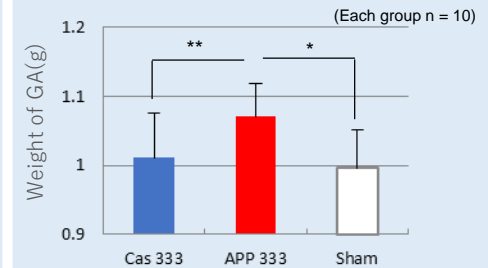
[Exp. 2] Orally administration of APP 333 mg/kg for 3 days significantly increased GA.

Fig. 4 Dose of APP orally administration (Each group n = 5)



Repeated oral administration: 6 weeks old male rats were orally administered with Cas and APP at 1000 mg/kg and 333 mg/kg for 3 days. After administration, the GA of rat were evaluated.

Fig. 5 Effect of APP 333mg / kg administration (Each group n = 10)



Repeated oral administration: 6 weeks old male rats were orally administered with Cas at 333 mg/kg and APP at 333 mg/kg, sham for 3 days. After administration, the GA of rat were evaluated.

In the APP 333 mg/kg group, mass of the gastrocnemius muscle increased significantly. Although there was no significant difference, the mean of gastrocnemius muscle mass in the APP 1000 mg/kg group showed a value close to APP 333 mg/kg. Fig.5 is shown that APP 333 mg/kg administration were increased significantly GA compared to Cas 333 mg/kg and sham administration. And **APP 333 mg/kg administration is minimum dose for increased GA**.

[Human effective dose (HED)] APP 53 mg/kg/day

$$\text{HED} = \text{animal dose in mg/kg} \left[\frac{\text{Animal weight in kg}}{\text{Human weight in kg}} \right]^{0.33} = 333 \text{ mg/kg} \left[\frac{0.19}{50 (*)} \right]^{0.33} = 53 \text{ mg/kg}$$

The human equivalent dose based on body surface area suggests that **53 mg/kg or more** may be effective. *: Average body weight of Japanese women (50 kg)

Table 2 Compositions of the experimental diets (g/1000 g diet).

	Cas	APP	A-APP	SPI	APP1/3	APP1/9
Casein	200	-	-	-	133	178
APP	-	182	-	-	59	20
A-APP	-	-	160	-	-	-
Soy been protein	-	-	-	208	-	-
L-Cysteine	3	3	3	3	3	3
α-Cornstarch	532	550	572	524	539	534
Sucrose	100	100	100	100	100	100
Cellulose	50	50	50	50	50	50
Soybean oil	70	70	70	70	70	70
AIN-93 mineral mixture	35	35	35	35	35	35
AIN-93 vitamin mixture	10	10	10	10	10	10
Total	1000	1000	1000	1000	1000	1000

% of crude protein	87.5	97.2	100.0	84.3	90.7	88.6
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In previous studies and all the experiments, diets are AIN-93G or protein source modified AiN-93G. Food intake is about 18-23 g/day. Alaska pollack fillets (Nippon Suisan Kaisha, Ltd., Tokyo, Japan) were used as the fish protein source in this study. The fillets were freeze-dried and ground. The fat component was extracted using hot ethanol (65° C, 60 min × 2) and removed through centrifugation. The remaining ethanol was dried in a vacuum dryer (60° C, ~24 h). The rats were housed in individual stainless wire mesh cages in a room under a 12 h light-dark cycle (dark phase: 15:00-3:00) at a constant temperature (22 ± 1° C). The animals were housed separately for 6 days for acclimatization to the environment. This study was conducted by March 2019 in accordance with the ethical guidelines of the Ehime University Animal Experimentation Committee and in complete compliance with the National Institutes of Health: Guide for the Care and Use of Laboratory Animals. All efforts were made toward minimizing the number of animals used and limiting experimentation to what was necessary to produce reliable scientific information.

[Conclusions] The increasing effect of APP on skeletal muscle was exerted in more **than one-third APP substitution in dietary administration**. Moreover, the effective amount of APP was **333 mg/kg or lower in repeated oral administration**. It suggested that a protein or peptide was involved for the skeletal muscle increasing effect of APP, not amino acid balance. In addition, considering the application to humans, the human equivalent dose based on body surface area suggests **that 53 mg/kg or more may be effective**. So, APP dose is 2.65 g for day for 50 kg body weight. In considering the variation in body weight, **we decided to dose of APP 4.5 g daily in the clinical trial**. This dose corresponds to about 30 g of Alaska pollack fillet daily.