Utilization of industrial food by-products in Hokkaido, Japan for the production of value-added foods with unique bioactive properties

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Abstract

A dwindling global food supply has heightened the need for full utilization of limited resources, including agricultural and marine-derived products. With the world population projected to exceed eight billion people by 2030, there will be significant increases in food demand over the forthcoming years. Some of our on-going projects are focused on maximizing the utilization of food products harvested in Hokkaido, Japan. This paper will first provide a brief overview of the mounting setbacks of increased food processing waste, termed by-products, followed by discussions on a novel value-added product development project focused on the production of nano-capsules derived from marine oil. The unique biofunctionalities of these compounds, as revealed by animal studies, will also be addressed. A better understanding of the favorable health effects of these natural products will benefit both the agricultural and seafood industries in Hokkaido and will assist their expansions into niche markets.

Keywords: bioactive peptide, by-product, marine carotenoid, nano-capsule, n-3 polyunsaturated fatty acid

Introduction

Global environmental problems are a growing concern in current society. One of the driving forces for these problems is considered to be population growth, which increases the demand for environmental resources such as agricultural and marine-derived foods. As food industrial by-products are a major source of environmental contamination, research has been carried out to develop methods to transform these by-products into valuable products (Bhaskar et al., 2007; Kristinsson and Rasco, 2000; Laufenberg et al., 2003). The basis of our research has been to convert these by-products into value-added functional foods or nutraceuticals. We are currently working on two major on-going projects focused on increasing food utilization

through food science and technology. Due to space limitations, only one of the two main topics introduced at the time of the OASERD conference will be addressed here. For further information, readers are referred to our previous publication (Liyanage et al., 2009) regarding effect of bioactive peptides derived from potatoes on lipid metabolism and cecal fermentation.

Anti-obesity effect of *Undaria* lipids nano-capsule prepared from scallop phospholipids

Background information

Approximately 30,000-40,000 tons of scallop processing by-products are generated annually in Hokkaido, Japan. Among these by-products is scallop viscera, which contains elevated levels of heavy metals and is considered to be a severe environmental hazard. Scallop viscera contain a high concentration of organic compounds such as proteins and phospholipids (PLs). PLs derived from scallops have high levels of long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids have been shown to reduce adiposity in animals fed a high-fat diet by limiting hypertrophy and hyperplasia of fat cells (Flachs et al., 2006). An *in vitro* study also revealed that DHA causes fat reduction by reducing preadipocyte differentiation, inducing apoptosis, and promoting adipocyte delipidation (Kim et al., 2006). Similarly to these bioactive fatty acids, our previous studies clearly demonstrated that consumption of *Undaria* lipids (ULs) containing the carotenoid fucoxanthin is associated with improved lipid metabolism and anti-obesity activity.

Nano-capsules are submicroscopic colloidal bioactive agent carrier systems composed of either a lipid-rich or an aqueous core surrounded by a thin polymer membrane. Owing to the presence of both lipid and aqueous phases, nano-capsules can be utilized in the entrapment, delivery, and release of both water-soluble and lipid-soluble material (Mozafari et al., 2008). Phospholipids such as soy lecithin have been the most utilized component for nano-capsule formation in food and drug delivery systems. In the current study, PLs derived from scallop by-products were used in the place of soy lecithin to produce nano-capsules. Given that both fucoxanthin and n-3 PUFA exhibit encouraging antiobesity activity, incorporation of ULs into n-3 PUFA-rich PLs might enhance lipid metabolism. Therefore, the aim of this investigation was first to develop a novel nanoencapsulation technique utilizing scallop-derived PLs with subsequent incorporation of ULs, and secondly to evaluate the antiobesity effect of these bioactive lipids in a nanoencapsulated form.

Animal care and diet/drink preparation

This study was conducted with 3-week-old male KK-A^{y} mice purchased from CREA Japan

(Tokyo, Japan). Mice were individually housed in plastic cages at a constant humidity (55%) and temperature (23 ± 1°C), with a 12-h light/dark cycle throughout the experiment and free access to drinking water or the experimental drink (i.e., mice were given the experimental drink instead of water). After a one-week acclimatization period with control diets, the mice were randomly divided into seven groups of seven mice each and fed either the experimental drink or diet for 4 weeks. The mice receiving the experimental drink continued to receive the control diet. The body weight of each mouse was recorded daily, as well as food and drinks/water intake.

At the end of the experimental period, the rats were fasted for 12 h, and blood samples were taken under inhalation anesthesia (diethyl ether) by cardiac puncture. The weight of organs and adipose tissue—both white adipose tissue (WAT) and brown adipose tissue (BAT)—were determined and expressed as grams per 100 g body weight. Experimental diets were prepared according to the recommendations of the American Institute of Nutrition (AIN-93G). The composition of the experimental diets and drinks remained the same for all groups, with the only variable being the type of lipid (*Undaria* lipid and/or phospholipids) (Table 1).

Table 1. Concentration of Undaria lipids and scallop midgut gland phospholipids in the experimental drinks or diets.

		Drinks			Diets		
		0.2%UL	0.3%PL	0.2%UL +0.3%PL	1%UL	1%PL	1%UL +1%PL
Diet (wt%)	Soybean oil	13.51	13.51	13.51	12.51	12.51	11.51
	Undaria lipid	0	0	0	1.00	0	1.00
	Scallop midgut gland phospholipid	0	0	0	0	1.00	1.00
Drink (wt/v, %)	Undaria lipid	0.20	0	0.20	0	0	0
	Scallop midgut gland Phospholipid	0	0.30	0.30	0	0	0

Determination of plasma lipid profile

At the time of dissection, blood samples were collected immediately and centrifuged (High-Speed Micro Centrifuge, HITACHI, Japan) at 1400 × *g* for 10 min. The samples were brought to the Hakodate Medical Association Inspection Center for plasma lipid composition

analysis. The analysis included measurements of the following parameters: triacylglycerols (TG), total lipids, and cholesterol (total, HDL, LDL, free) levels.

mRNA analysis

The WATs were dissected, washed with cold saline solution, and weighed. A portion of these adipose tissues were kept in RNA Later Storage Solution (Sigma Chemical Co., St. Louis, MO) at -20°C for future determination of UCP1 mRNA expression. Total RNA was extracted from these samples (>100 mg) using the RNeasy Lipid Tissue Mini Kit (Qiagen, Tokyo, Japan). cDNA was synthesized from total RNA utilizing the high-capacity cDNA archive kit (Applied Biosystems, Japan Ltd., Tokyo, Japan). The PCR solution was prepared by adding Syber Green PCR Master Mix solution (25 μL, Applied Biosystems), multiscribe reverse transcriptase (50 U/μL), RNase inhibitor (20 U/μL), template RNA, each primer at 200 nM, and RNase-free water. PCR primers, UCP1, and an internal control, mouse glyceraldehydes-3-phosphate dehydrogenase (GAPDH), were purchased from Applied Biosystems (Japan Ltd., Tokyo, Japan). The primer sequences used for detection of UCP1 and mouse glyceraldehyde-3-phosphate dehydrogenase (GADH; internal control) were as follows. Forward: 50CTCAGGATTGGCCTCTACGACTC30 and reverse: 50TTGGTGTACATGGACATCGCA30; for UCP1 and forward: 50GAAGGTCGGTGTGAACGGATT30 and reverse: 50GAAGACACCAGTAGACTCCACGACATA30 for GADH . Real time quantitative RT-PCR analysis was applied in an automated sequence detection system (7500-Real Time PCR System; Applied Biosystems Japan Ltd., Tokyo., Japan). PCR temperature cycling conditions were 50°C for 2 min, 95°C for 10 min, and then 40 cycles of 95°C for 15 s and 60°C for 1 min.

Western blotting analysis

After the dissection, WAT was rapidly frozen with liquid nitrogen and stored at -70°C for western blotting analysis. Each WAT (~500 mg) was homogenized by RNase-Free Disposable Pellet Pestles in a 250-mL buffer solution containing 10 mM Tris-HCl and 1 mM EDTA (pH 7.4) for 30 s with a Polytron. The mixture was then centrifuged at 1500 x *g* for 5 min, and the lower layer, containing soluble proteins, was obtained as fat-free extract. Total protein content in the extract was determined with a DC protein assay kit (Bio-Rad, Tokyo, Japan). Each sample solution was electrophoresed using a 10% SDS-polyacrylamide gel and approximately 40 mg of protein per lane. Following electrophoresis, the gels were transferred into buffer (25 mM Tris, 5% MeOH), and the proteins were subsequently blotted onto a PVDF membrane (AE-6677 ATTO; Tokyo Japan). After washing with PBS-Tween (PBS with 0.1% Tween 20), the primary antibody (UCP1; Sigma, St. Louis, USA; diluted 1:1000 was applied for 1 h

at room temperature. After five washes with PBS-Tween and four washes with blotto, a secondary antibody (rabbit IgG-conjugated horseradish peroxidase; Santa Cruz Biotechnology, Santa Cruz, CA, USA; diluted 1:2000 in blotto) was applied for an additional hour at room temperature. After another five washes with PBS-Tween followed by four washes with blotto, UCP1 was detected using a chemiluminescence detection kit (ECL system, Amersham Pharmacia Biotech, Piscataway, NJ, USA) following the manufacturer's recommendations. The expression of β-actin was also detected as an internal control using a β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

Results and Discussion

Changes in body weight

Figure 1 illustrates the changes in body weight recorded daily during the experimental period. The experimental drink groups that received either 0.2% UL or the nano-capsules (0.2% UL+0.3% PL) exhibited significant reductions in body weight during the experimental period, beginning at 15 days. These results indicate that the experimental drinks containing UL alone or with PL likely possess a certain degree of anti-obesity activity. For the groups fed experimental diets, there were no significant differences in body weight.

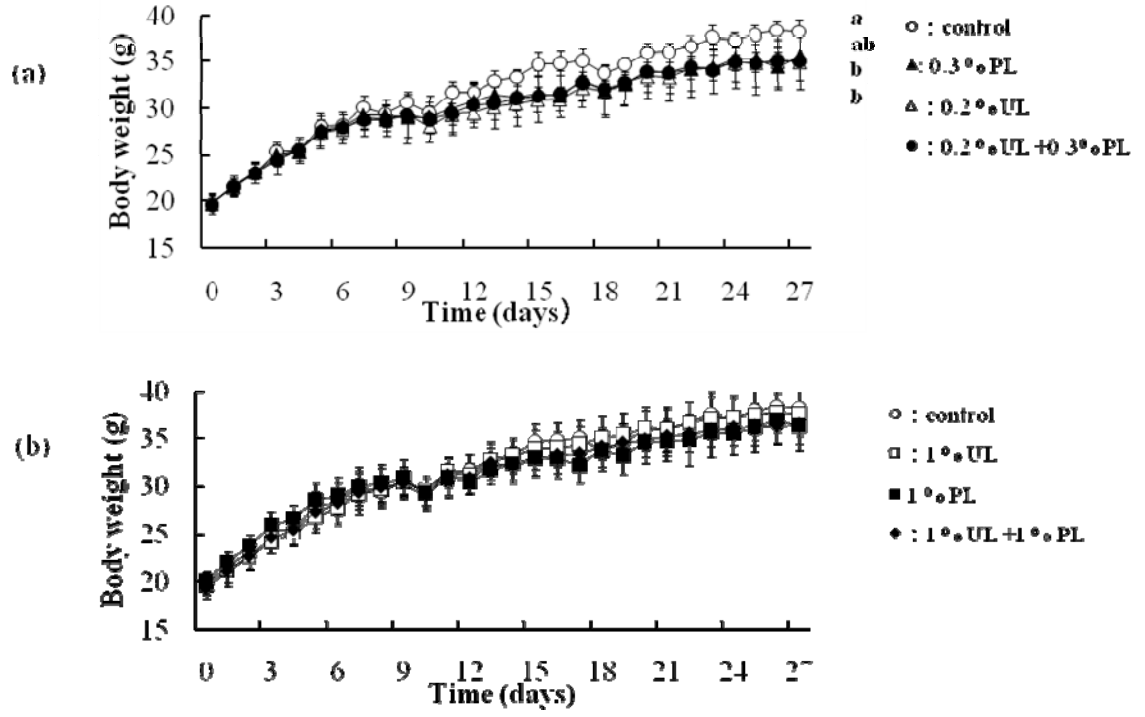

Figure 1. Changes in body weight of KK-A^y mice during the experimental period for (a) experimental drinks and (b) experimental diets. KK-A^y mice received either the 0.2% Undaria

lipid (UL) drink (△), 0.3% scallop midgut gland phospholipid (PL) drink (▲), 0.2% UL + 0.3 % PL drink (●), 1 % UL diet (□), 1 % PL diet (■), 1 % UL + 1% PL diet (◆) or the control diet (○). Groups that exhibited significant differences (at 4 weeks) are listed to the right of each graph, with a different superscript letter representing a significant difference (p < 0.05).

mesenteric WAT weight was reduced in all experimental groups as compared to the control group. This reduction was significantly different ($p < 0.05$) from the control group for mice receiving the nano-capsule drink (0.2% UL + 0.3% PL; 4.45 ± 0.73 g/100 g body weight), 1% UL diet (4.43 ± 0.27 g/100 g body weight) and 1% UL+1% PL diet (4.37 ± 0.53 g/100 g body weight). For total WAT that is composed of perirental WAT, retroperitoneal WAT, mesenteric WAT, epididymal WAT, and gluteal WAT, only the nano-capsule drink group (9.02 ± 1.23 g/100 g body weight) exhibited a significant weight reduction compared to the control group (10.1 ± 0.41 g/100 g body weight).

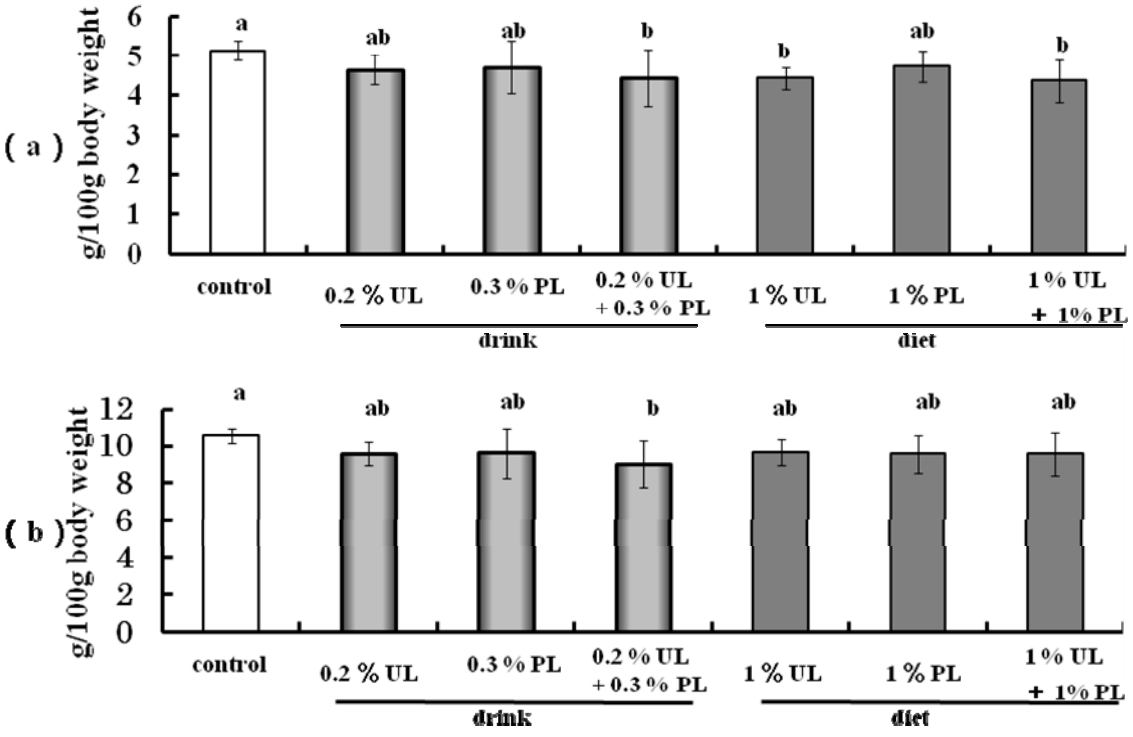

Figure 2. (a) Mesenteric white adipose tissue (WAT) weight and (b) total WAT weight of mice receiving 0.2% Undaria *lipid (UL) drink, 0.3% scallop midgut gland phospholipid (PL) drink, 0.2% UL drink + 0.3% PL drink, 1% UL diet, 1% PL diet, 1% UL + 1% PL diet, and the control diet. Columns with different superscript letters indicate a significant difference between treatment groups (p < 0.05).*

Serum lipid profiles

Both the 0.2% UL drink group and the 1% UL diet group exhibited higher concentrations of total cholesterol, HDL-cholesterols, and phospholipids as well as LDL-cholesterols (Table 3).

Table 3. Lipid parameters in the serum of KK-A^y mice fed with the experimental drinks or diets.

		drink			diet		
	control	0.2%WL	0.3%PL	0.2%WL +0.3%WL	1%WL	1%PL	1%WL +1%PL
Total cholesterol (mg/dL)	125±15^{a}	180±21^{b}	113±25^{a}	129±14^{a}	228±35^{c}	117±8.0^{a}	210±31^{c}
HDL cholesterol (mg/dL)	75.5±11^{a}	102±11^{bc}	76.9±18^{a}	86.3±9.8^{ab}	110±14^{c}	78.3±7.5^{a}	109±32^{c}
LDL cholesterol (mg/dL)	12±0.6^{abc}	15.1±2.7^{cd}	10.4±2.5^{ab}	10.7±1.4^{ab}	15.7±5.1^{d}	9.14±1.2^{a}	13.4±3.7^{bcd}
Neutral Fat (mg/dL)	135±39^{a}	170±55^{a}	134±49^{a}	121±26^{a}	146±65^{a}	161±67^{a}	138±41^{a}
Phospholipids (mg/dL)	228±29^{a}	298±23^{b}	284±38^{a}	227±27^{a}	322±43^{b}	209±12^{a}	299±31^{b}
FFA (μEq/L)	1276±358^{a}	1494±282^{a}	1288±273^{a}	1335±256^{a}	1318±309^{a}	1261±156^{a}	1197±219^{a}

Rows with different superscript letters indicate a significant difference between treatment groups ($p < 0.05$).

These results are in agreement with our previous study (Liyanage et al., 2009), which showed that incorporating UL into the diet leads to increased concentrations of these serum lipids (data not shown). On the other hand, feeding nano-capsules containing both 0.2 % UL and 0.3% PL only resulted in elevated levels of HDL-cholesterol (86.3 mg/dL) compared to the control group (75.5 mg/dL). In terms of total cholesterol concentrations, treatment with drinks or diets containing PL resulted in similar levels as the control group. On the other hand, all groups fed UL, with the exception of the nano-capsule group, exhibited higher total cholesterol concentrations (180-228 mg/dL) than the control group (125 mg/dL). There were no significant differences among groups for the other lipid parameters measured, including GOT and GPT (indicators of liver functionality), TG, and FFA concentrations.

Expression of UCP1 and UCP1 mRNA in epididymal WAT

UCP1 is generally only expressed in BAT, but can be expressed in other tissues such as WAT and muscle with an appropriate stimulus. The energy obtained from nutrients can either be dissipated as heat via UCP1 or used for ATP synthesis by the enzyme ATP synthase (Bray and Tartaglia, 2000). For this reason, up-regulation of UCP1 expressions in adipose tissues is linked to reductions in adipose tissue mass, leading to an encouraging anti-obesity effect.

This study demonstrated that diets containing 1% UL did not show significant anti-obesity

effects, probably due to the low concentration of fucoxanthin mixed in the diet. However, western blotting analysis revealed that the group receiving UL + PL nano-capsules demonstrated a significantly higher level of UCP1 expression (2.15 times higher than that of control group) (Fig. 3).

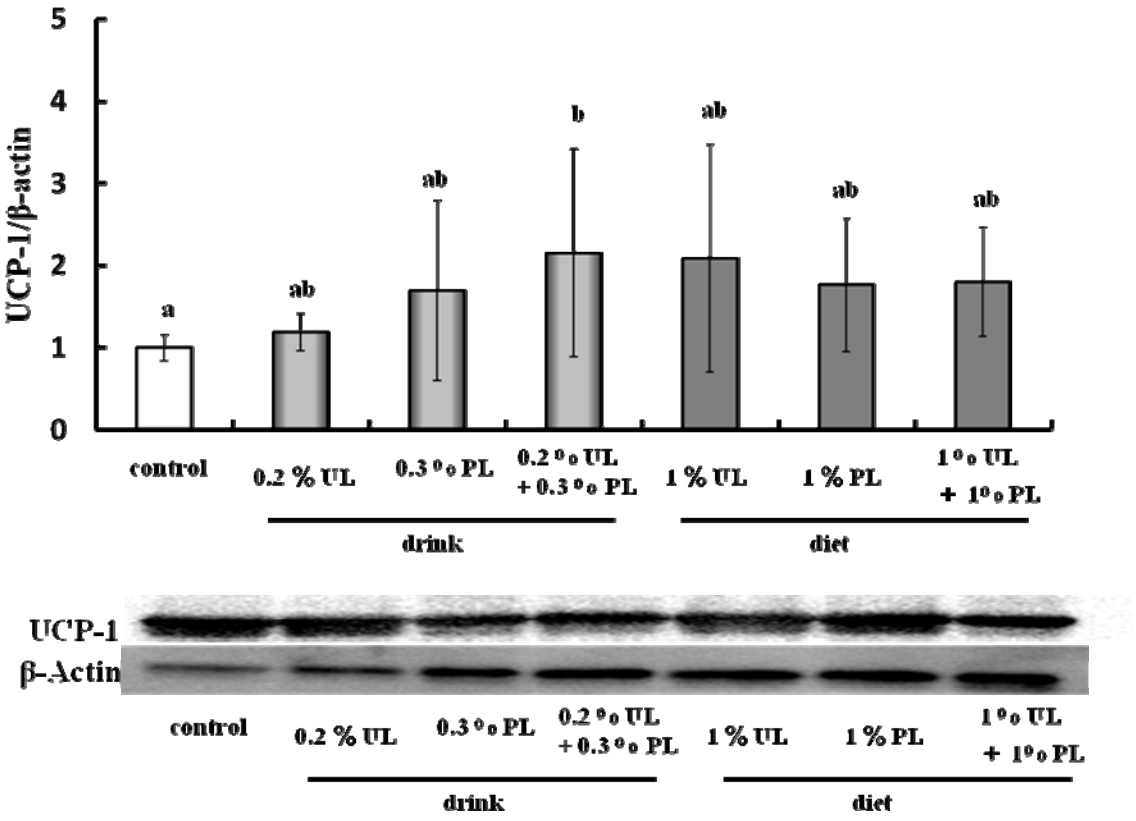

Figure 3. Western blotting analysis of uncoupling protein 1 (UCP-1) in epididymal WAT and relative expression level of UCP-1 protein compared to β-actin. Columns with different superscript letters indicate a significant difference between treatment groups ($p < 0.05$).

In order to determine the mRNA expression levels of UCP1, RT-PCR analysis was carried out for epididymal WAT (Fig. 4). Although the group receiving 0.2% UL and the nano-capsule group exhibited higher UCP1 mRNA expression levels than the control, these levels were significantly different only in the case of the nano-capsule group (4.23 times higher than that of control group). These results suggest that UL and PL work in a synergistic manner to reduce obesity, resulting in a greater anti-obesity effect than that which would be expected at these reduced concentrations.

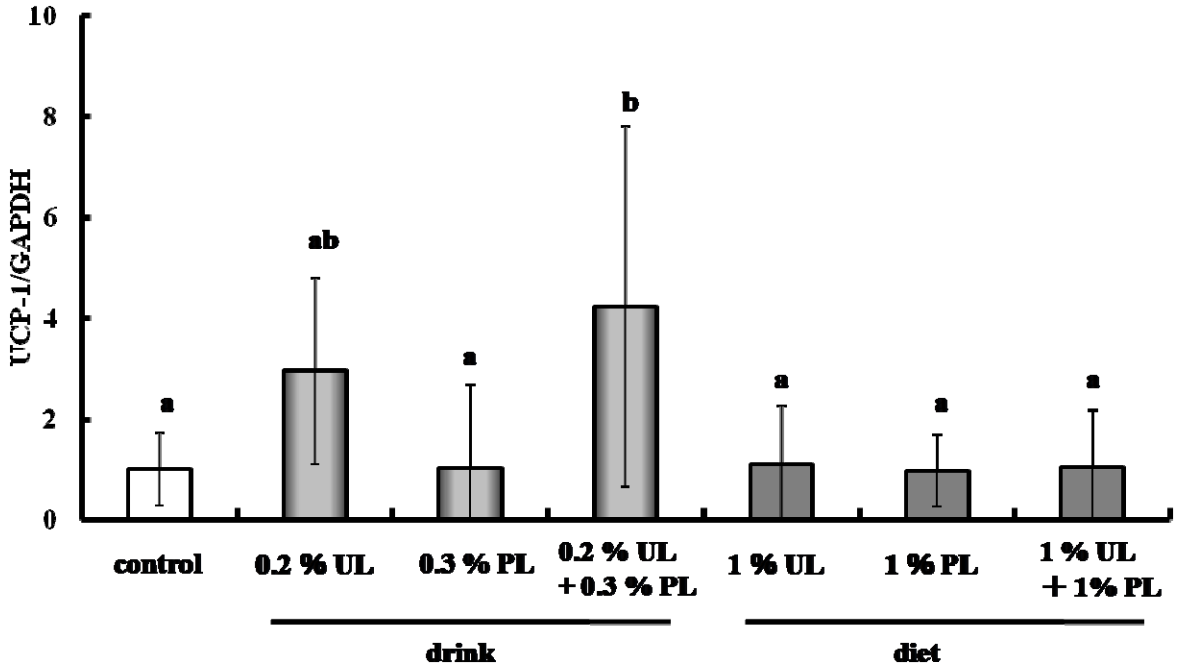

Figure 4. UCP-1 mRNA expression levels in epididymal WAT. Expression of UCP-1 mRNA was estimated by quantitative real-time RT-PCR. Relative values were presented as the ratio of UCP-1 mRNA to GAPDH mRNA. Columns with different superscript letters indicate a significant difference between treatment groups ($p < 0.05$).

Conclusions

In this study, a lipid delivery system was developed and tested for its anti-obesity effect in KK-A^y mice. Significant reductions in body weight and fat mass were observed in a diabetic-obese mice model by administering nano-capsules containing bioactive lipids. The combination of UL and PL was found to result in a synergistic effect, as compared to administrating either lipid alone. The observed reduction in body weight was likely due to increases in the expression of UCP1 and UCP1 mRNA found in epididymal fat tissue.

A better understanding of the precise mechanisms of weight reduction through UCP1 expression will likely lead to new approaches for managing obesity. In addition to the lipids utilized in this study, any hydrophilic compounds with anti-obesity effects could be incorporated into the nano-capsule delivery system developed here, leading to numerous potential applications. This study has shown that various bioactive agents can be utilized with combinative applications in order to increase energy expenditure and help to reduce body weight and fat mass.

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