Whiteleg shrimp shell powder ameliorates adiponectin and triglyceride-to-HDL ratio in type 2 diabetic rats

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Abstract

Purpose – Adiponectin, a bioactive molecule produced by adipose tissue, has potential effect in increasing insulin sensitivity. Adiponectin levels reduction is associated with type 2 diabetes mellitus (T2DM) and its complications, including cardiovascular disease (CVD). Triglyceride-to-high-density lipoprotein (TG:HDL) ratio can be used as a predictor of CVD risk in T2DM patients. Whiteleg shrimp (*Litopenaeus vannamei*) shell contains astaxanthin, macro- and micro-nutrients that may exert synergistic beneficial effects. This study aims to determine the effect of *L. vannamei* shell powder (LVSP) in improving adiponectin, TG, HDL and TG:HDL of T2DM Wistar rat, and to investigate the presence of any correlations between adiponectin and lipid markers.

Design/methodology/approach – A total of 25 male Wistar rats were divided into five equal groups: control negative [C(−)], control positive [C(+)], treatments 1, 2 and 3 (T1, T2 and T3, respectively). C(+), T1, T2 and T3 were maintained on a high-fat diet for 14 days before streptozotocin (STZ) injection. T1 and T2 groups were administered two different doses of LVSP, while T3 group was provided astaxanthin supplement (AST).

Findings – LVSP treatments significantly increase adiponectin ($p = 0.04$) and HDL ($p < 0.001$) but reduced TG ($p < 0.001$) and TG:HDL ($p < 0.001$). A higher LVSP dose was more effective in improving all markers than the lower dose; moreover, there was a comparable effect as that of AST in increasing the adiponectin levels. Strong correlations were observed between adiponectin and lipid markers.

Originality/value – This study shows that LVSP as a functional food, can ameliorate adiponectin levels and normalizes blood glucose levels. The LVSP reduces the risk of CVD because of the reduction of TG:HDL.

Keywords Adiponectin, Litopenaeus vannamei, TG-to-HDL ratio

Paper type Research paper

Introduction

Diabetes mellitus (DM) is a chronic, systemic disease associated with pancreatic-β-cells dysfunction accompanied by mass and cell function decrease, which impairs insulin function in regulating glucose, lipids and proteins metabolism (Skovso, 2014). Type 2

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diabetes mellitus (T2DM) is one of the four types of DM whose prevalence has significantly increased recently (Cho *et al.*, 2018). T2DM increases exponentially with the increase in body mass index (BMI) $>25\,kg/m^2$, depending on age, sex, duration and distribution of adipose tissue (Day and Bailey, 2011). Adipose tissue is considered both as an inert tissue that stores fat and as an active endocrine organ that produces adipokines. Adiponectin is one of the adipokines that has a potential role in increasing insulin sensitivity. Studies have shown that an increase in adipocytes and adipose mass both in human and in animal models is not directly proportional to the production of adiponectin and its receptors (Chakraborti, 2015).

Adiponectin is found as a trimer–dimer of low molecular weight (LMW) and a multimeric structure of high molecular weight (HMW) in the serum. The LMW form is primarily found in the circulation, whereas the HMW form constitutes a major portion of intracellular adiponectin and biologically acts as an antiatherogenic, antidiabetic and anti-inflammatory agent. Adiponectin has two types of receptors, including AdipoR1 that may be found ubiquitously, mostly in the skeletal muscle and AdipoR2 that is largely expressed in the liver. Both receptors are important in increasing adenosine monophosphate-activated protein kinase (AMPK) activity, peroxisome proliferator-activated receptor α (PPARα) ligand, fatty acid oxidation and glucose uptake (Caselli, 2014). Adiponectin also plays an important role in suppressing metabolic dysfunction, such as insulin resistance, T2DM, metabolic syndrome and cardiovascular disease (CVD).

T2DM condition is associated with lipid abnormalities and elevation of vascular complication (Bahlil *et al.*, 2018). The increase of triglyceride levels happened after insulin resistance disturbs glucose uptake and declines lipase sensitivity, thereby causing lipolysis and elevation of fatty acids in the circulation. These non-esterified fatty acids are partially converted into energy, while the excess gets accumulated in the liver and becomes triglycerides, resulting in the elevation of triglyceride levels (Vergès, 2015; Parashram and Lamlakar, 2016). Hypocatabolism of very low-density lipoprotein (VLDL) due to the disruption in the synthesis and activity of lipoprotein lipase (LPL) is also found in T2DM, which results in the accumulation of triglycerides in the circulation (Padalkar *et al.*, 2012; Vergès, 2015).

Hypertriglyceridemia in T2DM have also been to reported correlate with the decrease in the concentration and quality of high-density lipoprotein (HDL) (Wang and Peng, 2011). There are three types of mechanisms associated with this condition, which include the increase in HDL catabolism regulated by hepatic lipase in insulin resistance, the increase in VLDL production without any proportional catabolism and the decrease in plasma adiponectin levels (Vergès, 2015). Lipid abnormalities in T2DM also increase the risk of CVD complications by two or three times higher (Bahlil *et al.*, 2018), however, it can be predicted by triglyceride-to-high-density lipoprotein (TG:HDL) ratio. This index considered as more sensitive predictor for CVD risk in T2DM (Vega *et al.*, 2014).

Whiteleg shrimp *(Litopenaeus vannamei)* is one of the widely cultivated shrimp in Indonesia with a high demand for export. The majority (80-90 per cent) of the export activity is carried out in the form of headless frozen shrimp. Approximately 75 per cent of the total shrimp consists of shell and head, so that about 40-45 per cent of the entire shrimp body has been considered as a by-product (Suptijah *et al.*, 2011; Vázquez *et al.*, 2017). The shrimp shell contains nutrients, which is as good as the flesh. The primary components of the crustacean shell include chitin (15-40 per cent), protein (20-40 per cent), calcium and magnesium carbonate (20-50 per cent), and other micronutrients such as astaxanthin, lipids and minerals (Khoushab and Yamabhai, 2010).

Astaxanthin is a potent antioxidants that found highest in *Haematococcus pluvialis* (40 g/Kg dry biomass) and can help in prevention and treatment of diabetes, CVDs, metabolic syndrome and cancer (Sahni *et al.*, 2019). It has been reported that approximately 75 per cent of the total

carotenoids in whiteleg shrimp flesh contain astaxanthin (2.82 mg/kg of flesh) (Da Silva *et al.*, 2015). A dry-weight whiteleg shrimp shell contains higher astaxanthin amounts than the flesh (101.7 μg/g) (An *et al.*, 2008). To our knowledge, the effects of whiteleg shrimp shell powder with its internal components on the levels of adiponectin, HDL, triglycerides and TG:HDL ratio in diabetic Wistar rats have not yet been examined. Therefore, this study was conducted to determine the effect of *L. vannamei* shell powder (LVSP) in improving the levels of adiponectin, triglycerides, HDL and TG:HDL ratio in diabetic Wistar rats.

Method

Animal

A total of 25 male Wistar rats, eight weeks old, 150-200 g of body weight were purchased and kept in stainless steel cages with a 12 h light/dark period at a temperature of 20°C ± 1° C, in Laboratorium Hewan Coba, PSPG UGM, Yogyakarta, Indonesia. Rats were divided equally into five groups, control negative [C(−)], control positive [C(+)], Treatments 1, 2 and 3 (T1, T2 and T3, respectively). Each rat was given 15 g of standard feed (Table I) and an *ad libitum* water during acclimatization and treatment.

The protocol and the experimental design of the study were approved by the Ethics Committee, Medical Faculty, Diponegoro University, and Dr Kariadi Hospital Semarang, Indonesia (Ethical Clearance No. 118/EC/H/FK-RSDK/X/2018).

Litopenaeus vannamei shell powder preparation

L. vannamei shells (PT. Misaja Mitra, Tayu, Pati, Indonesia) were cleaned from dirt and the remaining flesh using running water and kept in a polyethylene bag in a freezer with the optimum temperature around −18°C-(−10)°C. The shell is dried using freeze-drying method (Laboratorium Mikrobiologi PAU, Universitas Gajah Mada, Yogyakarta) in −40°C for 3-4 days. Dried shells were crushed using a food processor and sifted using the 60-mesh sieve. The ratio of carapace:abdomen:thorax in each bottle was 1:1:1. The ready to use LVSP was stored in a dark bottle coated with aluminum foil outside and an oxygen scavenger inside and kept in a refrigerator at 4°C.

Type 2 diabetes mellitus induction

T2DM was induced in C(+), T1, T2 and T3 groups. The induction was performed after seven days of acclimatization by first administering 15 g of high-fat diet (HFD) per rat for 14 days after acclimatization. HFD consisted of a standard feed with the addition of lard to increase the cholesterol level by 1.25 per cent (Table I). Streptozotocin (STZ) (Nacalai Tesque, Kyoto, Japan) and nicotinamide (NA) (Nacalai Tesque, Kyoto, Japan) were injected

Nutrient	Standard feed (%)	HFD
Water	12	90% of standard feed + 10% of lard*
Crude protein	15	
Crude fat	3-7	
Fiber	6	
Ash	7	
Calcium	0.9-1.1	
Phosphor	0.5	

Note: *This composition may increase cholesterol level of the HFD up to 1.25 per cent

Table I.
The composition of standard feed and HFD

intraperitoneally after HFD phase. Each rat in the T2DM-induced groups received NA (110 mg/kg BB; diluted in a saline buffer) injection 15 min before the administration of STZ (45 mg/kg BB; diluted in a citrate buffer). T2DM developed after three days of injection. Rats with a fasting blood glucose level of >250 mg/dL were considered to have T2DM condition (Ghasemi *et al.*, 2014).

Treatment

Rats in the C(−) and C(+) groups received no treatment. LVSP was administered to the T1 and T2 groups at the doses of 0.89/200 g and 1.77/200 g of rat body weight, respectively. Astaxanthin supplement (AST) (ASTHIN® Force 4, SOHO, Indonesia) was provided to the T3 group at a dose of 0.09 mg/200 g of rat body weight. Both LVSP and AST were diluted in 0.5 per cent CMC–Na (Sigma-Aldrich, USA) till the solution became homogeneous. The intervention was administered for 21 days via oral gavage.

Body weight measurement

Rat's body weights were measured every week using a digital balance in the morning. The experimental animals were observed for signs of abnormalities and weight loss throughout the study period.

Blood sampling

Blood samples were collected twice, after T2DM induction and at the end of the intervention. The rats were fasted for 6-10 h before blood sampling. About ±3 ml of the blood collected from *plexus retro-orbital* was centrifuged at 4,000 rpm for 15 min to separate serum and platelets.

Determination of biochemical markers

Serum adiponectin levels were determined using enzyme-linked immunosorbent assay (ELISA) method. This method has been demonstrated to be a sensitive and selective method for evaluating antigens, proteins, peptides, nucleic acids and hormones by the principle of antigen-antibody reaction (Sakamoto *et al.*, 2018). The rat adiponectin ELISA kit (Fine Test, Wuhan, China) was used to measure the adiponectin levels.

Triglyceride levels were analyzed by the glycerol phosphate oxidase-p-aminophenazone (GPO-PAP) method. The principle of this method is that triglycerides are enzymatically hydrolyzed by LPL into glycerol and fatty acids. These fatty acids then react with glycerol kinase to form glycerol phosphate and convert into dihydroxyacetone phosphate and hydrogen peroxide by GPO. Hydrogen peroxide then react with 4-chlorophenol and 4-aminoantipyrine to form a colored 4 o-benzoquinone monomine complex and the absorbance can be measured by photometry. (Medichem Middle East, 2010). Determination of HDL levels was done by the cholesterol oxidase phenol 4-aminoantipyrine peroxidase (CHOD-PAP) method. The principle of this method is that when serum reacts with polyethylene glycol containing precipitating reagents, the entire VLDL and LDL are settled. The supernatant formed contains HDL, which thus becomes the sample to react with the CHOD-PAP reagent (Saikia *et al.*, 2016).

Triglyceride-to-high density lipoprotein measurement

The TG:HDL is an index that may be used as a predictor of CVD risk in T2DM patients (Vega *et al.*, 2014). This ratio can be calculated using the following formula:

$$TG:HDL = \frac{Triglyceride}{HDL}$$

Statistical analysis

The normality of the data were tested by the Shapiro–Wilk test. The significance between pre- and post-treatments of all parameters was analyzed by the paired *t*-test when the data were normally distributed and by the Wilcoxon test when the data were not normally distributed. Differences between the groups of rats were analyzed by one-way ANOVA (if the data were normally distributed and the variance was homogeneous) and by the Kruskal–Wallis test (if the data were not normally distributed). Correlations were evaluated using the Pearson correlation test (if the data were normally distributed) and the Spearman test (if the data were not normally distributed). Differences were considered to be significant when $p < 0.05$.

Result

T2DM induction increased the blood glucose level in the C(+), T1, T2 and T3 groups (420.8 ± 35.4 mg/dL). LVSP and AST treatments decreased the blood glucose level to 114.3 ± 18.1 mg/dL ($p < 0.05$) in the T1, T2 and T3 groups.

Body weight changes

The changes in the rats' body weight of the five groups showed on Table II. The mean body weight of the 25 rats before any treatment was 192.2 g ($p > 0.05$; homogeneous). Significant changes were observed in body weights in the C(+), T2 and T3 groups after HFD treatment compared to that in the C(−) group ($p < 0.05$). The highest increment in body weight was found in the C(+) group (51.8 ± 6.7 g; $p < 0.05$). STZ injection resulted in a significant decrease in the average body weights of C(+), T1, T2 and T3 groups compared with the T1 group that showed the most significant decrease after STZ injection (−16.6 ± 9.8 g; $p < 0.05$). LVSP and AST treatments significantly increased the body weight in the T1, T2 and T3 groups.

Adiponectin levels and its correlation with other biochemical markers

Adiponectin levels were significantly decreased in the T2DM-induced rat groups (C(+), T1, T2 and T3) compared to that in the C(−) group (Table III). Treatment with LVSP and AST significantly increased the levels of adiponectin in the T1, T2 and T3 groups and also exhibited a significant difference compared with the C(+) group (Table III). A higher dose of

Groups	Rats body weight (g)			
	Before HFD	After HFD	After STZ injection	After treatment
C(−)	203.8 ± 9.6	214.0 ± 7.3	220.2 ± 7.5	243.0 ± 10.4
C(+)	185.0 (184.0 – 201.0)	242.6 ± 11.4	236.4 ± 9.2	211.4 ± 9.0
T1	211.8 ± 13.0	225.0 ± 4.2	208.4 ± 6.4	228.4 ± 5.8
T2	194.4 ± 19.1	226.2 ± 20.0	217.0 ± 21.1	236.0 ± 22.1
T3	202.2 ± 13.4	228.4 ± 10.6	217.6 ± 12.0	236.6 ± 10.4

Notes: The data written as mean ± SD. Five groups of rats ($n = 5$ for each group) consist of C(−): control negative; C(+): control positive; T1: Treatment 1; T2: Treatment 2; and T3: Treatment 3

Table II. Changes of rats body weight over 46 days of experiment

Table III.
The effect of LVSP on adiponectin, triglyceride, HDL and TG:HDL

Marker \ Groups	C(−)	C(+)	T1	T2	T3
Adiponectin (mg/L)					
Pre	13.5 ± 0.4	4.9 ± 0.3	4.5 ± 0.3	4.4 ± 0.3	4.30 ± 0.38
Post	13.3 ± 0.4	4.4 ± 0.4	8 ± 0.6	11 (10.1 – 15.8)	12.6 ± 0.8
p	0.18	0.04	<0.01	0.04	0.04
Δ	−0.1 ± 0.2[b,c,d,e]	−0.6 ± 0.4[a,c,d,e]	3.4 ± 0.8[a,b,d,e]	7.3 ± 2.3[a,b,c]	8.7 (7.2 – 8.7)[a,b,c]
Triglyceride (mg/dL)					
Pre	71.4 ± 1.4	126.9 ± 4	118.7 ± 3	129.9 ± 1.2	127.6 ± 2.7
Post	74.3 ± 2.0	143.8 ± 4	128.0 ± 2.2	99.1 ± 2.6	90.4 ± 2.6
p	0.03	<0.001	0.01	<0.001	<0.001
Δ	3 ± 2.0[b,c,d,e]	17 ± 0.6[a,c,d,e]	9.3 ± 4.3[a,b,d,e]	−30.8 ± 2.1[a,b,c,e]	−37.2 ± 4.2[a,b,c,d]
HDL (mg/dL)					
Pre	80.4 ± 1.3	26.3 ± 1.6	29.2 ± 1.3	25.3 ± 0.8	24.1 ± 1.8
Post	77.7 ± 1.5	25.2 ± 1.2	49.0 ± 2.4	63.1 ± 2.3	70.1 ± 1.5
p	<0.001	<0.01	<0.001	<0.001	<0.001
Δ	−2.7 ± 0.4[b,c,d,e]	−1.1 ± 0.4[a,c,d,e]	19.8 ± 3.1[a,b,d,e]	37.7 ± 2.2[a,b,c,e]	46 ± 3.1[a,b,c,d]
TG:HDL					
Pre	0. 9 ± 0.0	4.8 ± 0.2	4.1 ± 0.2	5.1 ± 0.2	5.3 ± 0.4
Post	1 ± 0.0	5.7 ± 0.2	2.6 ± 0.1	1.6 ± 0.1	1.3 ± 0.0
p	<0.01	<0.001	<0.001	<0.001	<0.001
Δ	0.1 ± 0.0[b,c,d,e]	0.9 ± 0.1[a,c,d,e]	−1.5 ± 0.2[a,b,d,e]	−3.6 ± 0.1[a,b,c]	−4.0 ± 0.4[a,b,c]

Notes: Five groups of rats ($n = 5$ for each group) consists of C(−): control negative and C(+): control positive; T1: Treatment 1; T2: Treatment 2; T3: Treatment 3; *p*-value between pre- and post-treatment were analyzed using dependent *t*-test/wilcoxon; Δ: changes between pre- and post-value; differences between the groups of rats were analyzed using ANOVA (TG, HDL and TG:HDL)/Kruskal–Wallis (adiponectin); Alphabetical superscripts showed a significance level of [a]= $p < 0.05$ as compared to C(−); [b]= $p < 0.05$ as compared to C(+); [c]= $p < 0.05$ as compared to T1; [d]= $p < 0.05$ as compared to T2; [e]= $p < 0.05$ as compared to T3. The data were written as mean ± SD for normally distributed data and median (min-max) when the data were not normally distributed

LVSP administered to the T2 group resulted in better improvement in adiponectin levels than that achieved with the lower dose (T1), but no significant difference was found when compared with AST ($p > 0.05$). However, the best increase in adiponectin levels was observed in the T3 group.

The correlation analysis between adiponectin and other biochemical factors was done to find whether adiponectin levels relate to the blood glucose, TG and HDL levels, and TG-to-HDL ratio as a predictor of CVD in T2DM rats (Table IV).

Triglyceride levels

As shown in Table III, significant elevations in TG levels were observed after T2DM induction compared to that in the C(−) group. Both LVSP and AST treatments could significantly decrease the levels of triglycerides in the T2 and T3 groups to normal values (<100 mg/dL) (Rajiv *et al.*, 2013; Guo *et al.*, 2018; Vatandoust *et al.*, 2018). The best decrease in triglyceride levels was observed in the T3 group (Table III), but there was also a significant lowering effect of the treatment in the T2 and T3 groups.

High density lipoprotein levels and triglyceride-to-high density lipoprotein ratio

Significant reductions in HDL levels below the normal level 40 mg/dL (Khan and Makki, 2017; Guo *et al.*, 2018) were observed in the T2DM-induced groups (Table III). Treatment with LVSP and AST for 21 days was able to significantly enhance the HDL levels ($p < 0.05$) compared to that in the C(+) group (Table III). The increase in HDL levels in the T2 group was significantly better ($p < 0.05$) than that in the T1 group, although the value was still lower than that in the T3 group.

The TG:HDL showed an improvement after the treatments, which was in parallel to the improvement in triglyceride levels and HDL levels independently. The C(+) group rats showed the highest final TG:HDL ratio (5.7 ± 0.2; Table III). The decreases in the TG:HDL value in the T1, T2, and T3 groups pre- and post-treatment were found to be significant, although the values were still larger than the value in the C(−) group (Table III). The best decline was observed in the T3 group.

Discussion

This study demonstrated both increasing and decreasing patterns of body weight changes in the T2DM-induced Wistar rats (Table II). Previous studies have demonstrated that the higher increase in body weight in the HFD-fed group than that in the standard diet-fed group was because of differences in the individual response to the rate of weight gain. Several factors such as modulation of the lipogenesis gene, the amount of feed consumed and palatability of the feed are known to influence the increase in body weight in the HFD-

Y axis \ X axis	Triglyceride[a]	HDL[b]	TG:HDL[c]	Blood glucose[d]
Adiponectin	$r = -0.896$ $p < 0.001$	$r = 0.877$ $p < 0.001$	$r = -0.887$ $p < 0.001$	$r = -0.9$ $p < 0.001$
Blood glucose	$r = 0.975$ $p < 0.001$	$r = -0.975$ $p < 0.001$	$r = 0.984$ $p < 0.001$	

Notes: Spearman correlation test result of (i) Adiponectin with (a) Triglyceride, (b) HDL, (c) TG:HDL and (d) Blood glucose; (ii) Blood glucose with (a) Triglyceride, (b) HDL and (c)TG:HDL

Table IV. Correlation studies of adiponectin and blood glucose with another biochemical markers

fed animals (Rodrigues *et al.*, 2015). This mechanism may explain the lack of a statistically significant increase in the body weight of the T1 group although all groups received the same amount of HFD (15 g per rat). In this study, lard was added to the standard feed to increase the cholesterol level. Lard contains saturated fat that can increase fat deposits in the body, especially in the intra-abdominal region (Viggiano *et al.*, 2016). Experimental research has shown that rat groups fed with lard-HFD diet accumulated more total fat (32 per cent), visceral fat (30 per cent) and subcutaneous fat (36 per cent) than groups fed with vegetable fat, resulting in a significant increase in rat body weight (Kubant *et al.*, 2015).

T2DM induction using HFD and STZ has two synergistic goals, the development of insulin resistance in the HFD feeding phase and mild-level pancreatic β-cell dysfunction after STZ injection (Skovso, 2014). STZ is a T2DM-inducing agent that enters the rat body via GLUT-2 and causes DNA methylation and activation of poly-ADP-ribose polymerase 1 (PARP-1), thereby decreasing ATP formation and protein synthesis, as well as inducing pancreatic β-cell necrosis (Ghasemi *et al.*, 2014). T2DM that develops after STZ injection leads to disturbances in physiological metabolisms, such as weight loss and an increase in appetite and thirst as compensation for ATP and glycogen decline. The decline in ATP and glycogen levels is detected by the body as a signal of the absence of carbohydrates as an energy source, so that the body attempts to fulfill the ATP need by increasing the demolition of fat and muscle mass to be used as raw material for glucose formation through gluconeogenesis. Insulin resistance leads to failure of the body to store glucose in the form of glycogen, causing the body to continuously send a lack-of-glucose signal inducing significant weight loss (Bhuvaneswari and Anuradha, 2012; Al-attar and Alsalmi, 2019). Improvements in blood glucose levels after the intervention with LVSP and AST also have an impact in improving metabolism, marked by a decrease in the activity of glucose-6-phosphatase and fructose-1-6-bisphosphatase, which participate in glucose formation and decrease gluconeogenesis that has previously occurred in T2DM. The reduction of tissue protein disassembly as a raw material for gluconeogenesis results in improvement of weight gain and insulin sensitivity (Pournaghi *et al.*, 2012; Mahmoud *et al.*, 2017).

The collaborative research of this study found that LVSP treatment improved blood glucose levels and homeostatic model assessment of insulin resistance (HOMA-IR) (Ahriyasna *et al.*, 2019). The strong correlation observed between adiponectin and blood glucose levels (Table IV) is consistent to the study on rats with T2DM conducted in Egypt ($r = -0{,}3$; $p < 0.05$) (Eissa *et al.*, 2017), indicating that when the T2DM condition is restrained, characterized by the decline in blood glucose levels, adiponectin levels are increased and provide a reciprocal effect to blood glucose levels. The improvement of blood glucose levels after LVSP treatment also related to the improvement of TG, HDL and TG: HDL as shown in the correlation study in Table IV.

There are several ingredients in LVSP that may affect adiponectin levels. Astaxanthin, a potent antioxidant found in *L. vannamei* shells, is known to act as an anti-inflammatory agent through activation of a series of mechanisms, including rapid phosphorylation of insulin receptor (IR) and its substrates (IRS-1 and IRS-2), association of phosphatidyl inositol 3 kinase (PI3K) with IRSs and Akt activation (IRS-PI3K-Akt pathway). These mechanisms develop after the prevention of serine/threonine phosphorylation into IRS-1 and IRS-2 along with the down-regulation of the extracellular signal-regulated kinase (ERK-1) and c-jun N-terminal kinase (JNK-1) (Bhuvaneswari and Anuradha, 2012). A decrease in ERK-1 and JNK-1 activity is related to the antioxidant effects of astaxanthin, which can ameliorate oxidative stress, lipotoxicity and inflammation (Bhuvaneswari *et al.*, 2010), thereby decreasing reactive oxygen species (ROS) formation and increasing adiponectin production.

Adiponectin is known as a factor that contributes to lipid metabolism through PPARα. This study demonstrated a negative correlation between adiponectin and TG levels (Table IV). Astaxanthin because of its ability as an antioxidant can increase the concentration of adiponectin and reduce the amount of visceral fat in the body. The increase of Adiponectin can thus stimulate AMPK and activate PPARα. AMPK stimulation results in a decrease in plasma glucose levels by increasing glucose uptake. Both AMPK stimulation and activation of PPARα simultaneously increase fatty acid oxidation through the expression of carnitine palmitoyltransferase (CPT-1), which can facilitate the transfer of acyl-CoA long-chain fatty acids into mitochondria (Abranches *et al.*, 2011; Hardie, 2011; Mashhadi *et al.*, 2018). This mechanism prevents the excessive formation of energy from fatty acids so that triglyceride levels are suppressed.

In this study, the increase of adiponectin levels correlated to the increase of HDL levels (Table IV). The underlying mechanism might be explained through a previous study. The administration of astaxanthin increases adiponectin levels, and thus, activates PPARα. The activation of PPARα promotes LPL expression and increase its activity by stimulating apoliporpotein A-V (LPL activator) and reducing Apolipoprotein C-III (LPL inhibitor). This mechanism increase HDL cholesterol and promote cholesterol efflux from cells to HDL, mediated by stimulation of expression of the ATP-binding cassette A1 transport protein (Rigano *et al.*, 2017).

The administration of LVSP in its whole form does not eliminate other nutrients contained in the *L. vannamei* shell. Other ingredients may also play a synergistic role in improving the biomarkers in this study. Chitin and its derivative, chitosan, have a function like a fiber and exert antioxidant activities that are beneficial in reducing blood glucose levels. Chitosan can relieve oxidative stress and prevent lipid oxidation. In addition, chitosan is known to be able to increase glucose uptake signaling in the muscle through Akt phosphorylation and GLUT-4 translocation from the cytosolic membrane to the membranes in T2DM rat muscles (Liu *et al.*, 2010; Chi *et al.*, 2015; Rajalakshmi *et al.*, 2013).

Calcium in the form of calcium carbonate is the mineral with the highest amount (44.75 per cent) in shrimp shells. It is believed that calcium may be beneficial in increasing adiponectin levels indirectly through the improvement of inflammation under T2DM conditions (Mahmoud *et al.*, 2007; Tabesh *et al.*, 2014). The content of zinc (Zn) is known as much 13.3 mg/kg in dried shell and head of *Pandalus borealis* (Adeyeye *et al.*, 2016) and is expected to play a synergistic role in increasing adiponectin levels. In an earlier study, zinc supplementation was provided to human subjects with severe excess body (BMI $> 25\ kg/m^2$), which resulted in a significant increase in adiponectin levels ($p < 0.05$), although the decrease in the HOMA-IR index was not statistically significant (Soheilykhah *et al.*, 2012).

Sulfated glycosaminoglycan (GAG) is a by-product of shrimp shell processing obtained from the liquid phase and carotenoprotein residue is recovered after being extracted using ethanol and by centrifugation. Research has shown that the GAG content isolated from 0.236 kg of *L. vannamei* head was 79 ± 2 mg (Cahú *et al.*, 2012). Sulfated GAG is known to have a potential role as a powerful antioxidant that can play a significant role in reducing DPPH radicals and preventing lipid peroxidation (Sayari *et al.*, 2018). The content of GAG in the *L. vannamei* shell is believed to help reduce ROS, which suppresses adiponectin production under T2DM conditions, which is consistent with the increase in adiponectin levels after LVSP treatment in this study.

The lipid content in shrimp shells is generally dominated by monounsaturated fatty acids (MUFAs) in the range of 40.6-49.9 per cent (study of *Pandalus borealis* shrimp shells) (Jiao *et al.*, 2015; Adeyeye, 2017), followed by polyunsaturated fatty acids (PUFAs) as much as 29.2 per cent, with the majority of this PUFA consisting of omega-3 (25.2 per cent) and

omega-6 (1.7 per cent) fatty acids (Jiao *et al.*, 2015). Another study on the oil obtained from krill supplementation demonstrated that MUFA and PUFA had a good effect on weight loss and increased lipid metabolism, including increasing the HDL level (Vigerust *et al.*, 2013).

The TG-to-HDL ratio index is considered to be a sensitive predictor of the risk of CVD as it describes the presence of the metabolic syndrome that links T2DM with CVD (Vega *et al.*, 2014). Consistent with the previous statements that the shell of *L. vannamei* contains various substances (astaxanthin, chitin, chitosan, calcium, GAG, PUFA and MUFA) that may play an important role in ameliorating TG and HDL level in T2DM rats. The overall mechanism indicates that the substances present inside LVSP have a better effect in increasing the adiponectin levels and normalizing blood glucose levels, so that the TG:HDL being reduced, as well as the risk of CVD. The LVSP may act as a functional food with a comparable effect as that of AST.

Conclusion

LVSP ameliorates the levels of adiponectin, TG, HDL, and TG:HDL in T2DM-induced Wistar rats. The dose of 1.77/200 g rat body weight of LVSP has a better impact on all biomarkers. LVSP showed comparable effectiveness to the AST on improving adiponectin levels of T2DM animal models. The strong correlation between adiponectin and other biochemical markers indicated that the improvement of adiponectin related to the reduction of TG:HDL and the risk of CVD.

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