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Cardioprotective Modulation of Cardiac Adiponectin and Adiponectin Receptors by Omega-3 in the High-Fat Fed Rats

Sandra M. Younan[1], Leila A. Rashed[2], and Osama M. Abd El Aziz[3]

[1]*Department of Physiology*
[2]*Department of Biochemistry*
and
[3]*Department of Pediatric, Faculty of Medicine, Cairo University, Cairo, Egypt*

Abstract

Obesity is an important risk factor for heart disease. This study investigated the effects of omega-3 (ω-3) on reversal of high fat (HF) diet-induced changes in the expression of the cardiac adiponectin and adiponectin receptors R1 and R2. Male rats were fed low-fat (LF; 10% energy from fat) or HF (45% energy from fat) for 16 weeks, LF-ω-3 or a HF-ω-3 (LF or HF for 16 weeks supplemented by ω-3 as 36 g/kg diet for the last 6 weeks, respectively) and a HF diet for 10 weeks to demonstrate HF effect before ω-3 administration. HF diet induced obesity, glucose intolerance, increased heart end systolic and diastolic volumes, decreased serum adiponectin, reduced expression of cardiac and adipose tissue adiponectin and adipo R1 & R2 with elevated serum tumour necrosis factor-α (TNF-α) compared to the LF diet. On the other hand, the HF-ω-3 group compared with the HF group had improved glucose tolerance (area under the glucose curve 837.14 ± 45.7 versus 1158.5 ± 69.8) and insulin resistance with a significant increase in serum adiponectin (4.22 ± 0.39 versus 2.82 ± 0.69 ng/ml) and a significant decrease in serum TNF-α (129.84 ± 13.63 versus 209.8 ± 16.42 pg/ml) and triglycerides independent of obesity. Also the data showed significant increases in the expression of cardiac and adipose tissue adiponectin and adiponectin R1 and adipose tissue adipo R2 as well as cardiac pAMP kinase with improvement in end-systolic and -diastolic volumes. These parameters were also improved compared to initial values in HF-10-week group. In conclusion, dietary ω-3 supplementation has a beneficial effect on fat-induced cardiac dysfunction and insulin resistance partly through increasing adiponectin and adiponectin receptors expression in heart and adipose tissue.

Key Words: adiponectin, cardiac adiponectin receptors, omega-3-high fat diet

Introduction

Obesity is a major health problem associated with a high prevalence of obese subjects developing type 2 diabetes and cardiovascular diseases. In recent years, evidence has linked these disorders in part to adipose tissue endocrine functions (14). Indeed, adiponectin is an adipokine secreted by adipose cells that increases fatty acid oxidation and potentiates insulin inhibition of hepatic gluconeogenesis, thus promoting insulin sensitivity (2). Adiponectin plasma level has been reported to be significantly reduced in obesity (46).

Two adiponectin receptor genes have been cloned: adipo R1 is abundantly expressed in the skeletal muscle and adipo R2 is mostly abundant in the liver where it has an intermediate affinity for adiponectin (45). Although exclusive expression of adiponectin in adipose tissues was previously reported (27), Ding *et al*. (10) found that adiponectin and adipo R1/R2 were expressed in cultured normal rat cardiomyocytes where adiponectin exerted a role in

Corresponding author: Sandra Mourad Younan, 28 street 213-Maadi-Cairo, Egypt. Tel: +201000-108789, Fax: +202-24560282, E-mail: hazembeshay@yahoo.ca
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cardiac energy metabolism linked to the activation of adenosine monophosphate-activated protein kinase (AMPK). However, the expressional regulation of adiponectin and its receptors in cardiomyocytes, especially in obesity, remains to be defined.

There is extensive evidence in support of the concept that a high intake of omega-3 (ω-3) polyunsaturated fatty acids (ω-3PUFA) from fish oil, specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), exerts cardioprotective effects in terms of coronary artery disease and sudden cardiac death (30, 35). Most reports with fish oil supplementation indicate an up-regulation of the expression and secretion of adiponectin in the adipose tissue (11); however, there are also reports where adiponectin is unaffected by fish oil supplementation (8, 37). Little is known about ω-3PUFA impact on cardiac adiponectin expression especially in established obesity. The aim of this study was to investigate cardiac expression of adiponectin and its receptors R1 and R2 in a model of high-fat diet-fed rats and the possible impact of ω-3 supplementation.

Materials and Methods

Experimental Animals and Design

Male Wistar rats aged 4 weeks and weighing 120-130 g were purchased from the animal care unit of the Faculty of Medicine, Cairo University, and all procedures were approved by this unit. Rats were each housed in cages in a temperature (22-24°C)- and light-controlled room on an alternating 12: 12 h light-dark cycle. Except when scheduled for glucose tolerance test, all animals had free access to food and water. Animals were divided into the following groups (n = 7 per group):

LF group: Rats receiving a low-fat diet (LFD) (10, 20 and 70% of energy from fat, protein and carbohydrate, respectively) for 16 weeks.

LF-ω-3 group: Rats receiving LFD for 10 weeks and then LFD with ω-3 (Pharmavite, LLC, Northridge, CA, USA) for 6 more weeks.

HF group: Rats receiving high-fat diet (HFD) (45, 20 and 35% of energy from: fat (lard and corn oil) as 207 and 29 g/kg diet, respectively), protein and carbohydrate, respectively). This group was further divided into HF-10-week group to assess established HFD-induced changes after 10 weeks on measured parameters (as a control before starting diet intervention) and a HF-16-week group receiving HFD for 16 weeks.

HF-ω-3 group: This group of rats received a HFD for 10 weeks followed by HFD combined with ω-3 replacing the caloric equivalent of the lard-derived energy for 6 more weeks (lard, corn oil and EPA/ DHA in a 5:1 ratio as 171, 29 and 36 g/kg diet, respectively) (19).

Assessment of Cardiac Function

The echocardiogram was performed as described before (33). In this study, an echocardiograph color system Hewlett-Packard 5500 SONOS (HP, Palo Alto, CA, USA) ultrasonic machine equipped with a 12 MHz electronic-phased array transducer was used. Under intraperitoneal ketamine and xylosine (66.6 and 13.3 mg/kg respectively) anesthesia, the chests of the animals were shaved, and they were maintained either in left lateral decubitus or supine position. Images were obtained from the left parasternal and apical windows. Short-axis two-dimensional views of the left ventricle (LV) were taken at the level of the papillary muscles to obtain the M-mode recordings. Interventricular septum and posterior-wall thickness, end-diastolic and end-systolic LV internal dimensions were measured. The systolic function was expressed by the ejection fraction (EF), calculated by Simpson's method, after left ventricular volume calculation.

Intra-Peritoneal Glucose Tolerance Test (IPGTT)

At the end of the study, the rats were subjected to IPGTT, which was performed on the unanesthetized animal after 12 h fasting, as described previously (22). Immediately after the collection of a tail blood sample for fasting glucose, insulin, triglycerides (TG) and free fatty acids measurements, a glucose solution (2 g/kg) was given intra-peritoneally to each rat, and then serial blood samples from the tail vein were collected at 30, 60, 90 and 120 min after glucose administration for measurement of serum glucose levels.

Assessment of Obesity

To assess obesity, the body mass index (BMI) was calculated using the formula: weight in kg/body naso-anal length in m^2 (17).

Biochemical and Hormonal Assays

Fasting serum glucose was measured by the oxidase-peroxidase method using the spectrophotometer (Du 7400, Beckman Coulter Inc., CA, USA). Commercially available ELISA kits were used to measure serum insulin (Linco Research, St Charles, MO, USA), total adiponectin (B-Bridge International Inc., Monroe, Washington, USA) and TNF-α (Quantakine High Sensitive, R&D Systems, Minneapolis, MN, USA) using ELISA reader (Stat Fax-

2100 Awareness Technology Inc., Palm City, FL, USA). To estimate insulin resistance, the homeostasis model assessment for insulin resistance (HOMA-IR: insulin resistance index) was used, calculated as the product of fasting insulin (in μU/ml) and fasting glucose (in mM) divided by 22.5, which has previously been used in rodents (41). Serum TG was measured (Biovision, Mountain View, CA, USA) and its absorbance was measured spectrophotometrically at 570 nm.

Real-Time Quantitative Analysis of mRNA Levels of Adiponectin and Adiponectin Receptors

Under anesthesia, rats were decapitated, epididymal and retroperitoneal fats and the heart were dissected for determination of expression of the adiponectin and the adiponectin receptors, adipo R1 and adipo R2, genes. Total RNA was extracted from myocardial and adipose tissue homogenates with the RNeasy purification kit (Qiagen, Valencia, CA, USA). cDNA was generated from 5 μg of total RNA extracted with 1 μl (20 pmol) antisense primer and 0.8 μl superscript AMV reverse transcriptase for 60 min at 37°C.

Expression levels of mRNA for adiponectin, adipo R1 and adipo R2, were quantified by real-time RT-PCR using the SYBR Green chemistry (Applied Biosystems, Foster City, CA, USA) on an ABI prism 7500 sequence detector system. PCR primers were designed with Gene Runner Software (Hasting Software, Inc., Hasting, NY, USA) from RNA sequences from the GenBank. The adiponectin gene forward primer was: 5'-GAAGTAGACTCTGCTGAGATGG-3' and reverse primer was 5'-TATCAGTGTAGGAGG TCTGTGATG-3'; adipo R1 forward primer was 5'-GATTTTCCATGTCCTGGTGG and reverse primer was 5'-AGGCTCAGAGAAGGGTGTCA-3'; adipo R2 forward primer was 5'-TCTTCCTGTGCCTGGGG ATCTT-3' and reverse primer was 5'-CCCGATAC TGAGGGGTGGCAAA-3'. All primer sets had a calculated annealing temperature of 60°C. Quantitative RT-PCR was performed in duplicates in a 25-μl reaction volume consisting of 2X SYBR Green PCR Master Mix (Applied Biosystems), 900 nM of each primer and 2 μl of cDNA. Amplification conditions were 2 min at 50°C, 10 min at 95°C and 40 cycles of denaturation for 15 s and annealing/extension at 60°C for 1 min. Data from the real-time RT-PCR assays were calculated using the V 17 Sequence Detection Software from PE Biosystems (Foster City, CA, USA). Relative expression of the adiponectin, adipo R1 and adipo R2 genes was calculated using the comparative C_t method as previously described (24). All values were normalized to the GAPDH housekeeping gene: forward primer 5'-TCCCACTCTTCCACCTTC-3' and reverse primers 3'-CTGTAGCCGTATTCA TTGTC-5'.

Measurement of Adiponectin in Heart and Adipose Tissues

30 mg of each of the heart and adipose tissues was homogenized in 1 ml lysis buffer (0.0625 mol/l Tris buffer, pH 6.8, 2% sodium dodecyl sulphate (SDS), 3% 2-mercaptoethanol, 10% glycerol, 10 μ/ml aprotinin and 1mmol/l phenyl methyl sulphonyl fluoride [Sigma, St. Louis, MO, USA]) and centrifuged at 8,000 rpm for 20 min at 4°C. Adiponectin in the homogenates was measured using enzyme-linked immunosorbent assay using a commercial ELISA kit (Quantikine R&D system, Inc. Minneapolis, MN, USA).

Western Blot Analysis

Expression of the phosphorylated AMP-activated protein kinase (pAMPK) in the heart was determined as previously described (5). Heart tissue (50 mg) was homogenized using a polytron homogenizer in 1.5 ml cold lysis buffer (50 mM Tris-HCL, pH 8.0, 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS and 0.5 mM phenylmethylsulfonylfluoride). The homogenate was centrifuged for 20 min at 4°C and the supernatant was collected. Samples were stored at -80°C until use. After boiling at 95°C for 5 min, samples (50 μg/lane) were subjected to 7% SDS-PAGE gel and then transferred to polyvinylidene difluoride membrane (AMPK; Millipore, Bedford, MA, USA). The membranes were blocked in 7.5% non-fat dried milk in TBST (0.05% Tween-20 Tris-buffered saline) for 2 h at room temperature and then incubated with primary antibodies overnight at 4°C with phospho-specific antibodies against AMPK Thr^{172} at 1:1000 (Cell Signaling Technology, Beverly, MA, USA). The membranes were washed and then incubated with a secondary horseradish peroxidase-conjugated anti-rabbit IgG antibody (1:25 000, Bio-Rad, Hercules, CA, USA) for 1 h at room temperature, followed by additional washing. Proteins were visualized by enhanced chemiluminescence (ECL plus; Amersham, Arlington Heights, IL, USA) and quantified using densitometry and Molecular Analyst Software (Bio-Rad, Richmond, CA, USA).

Statistical Analysis

The results were analyzed using SPSS computer software package, version 10.0 (Chicago, IL, USA). Data were presented as means ± S.D. Differences among the parameters of the three groups were

Table 1. Effects of high-fat diet and omega-3 on the metabolic parameters

	LF	LF-ω-3	HF-10-wk	HF-16-wk	HF-ω-3
Initial Body Mass Index (kg/m^2)	3.71 ± 0.26	3.47 ± 0.12	3.51 ± 0.18	3.50 ± 0.20	3.48 ± 0.21
Body Mass Index at the End of the Study (kg/m^2)	5.57 ± 0.05	5.89 ± 0.44	6.40 ± 0.14*	7.70 ± 0.60$^{*,\#}$	7.00 ± 0.80^{+}
Fasting Serum Glucose (mM)	5.20 ± 0.30	4.98 ± 0.1	7.84 ± 0.4*	8.04 ± 0.6*	6.06 ± 0.50$^{+,@}$
Serum Insulin (μU/l)	8.15 ± 0.60	6.95 ± 0.73	16.30 ± 1.41*	18.30 ± 2.32$^{*,\#}$	10.39 ± 1.78$^{+,\#,@}$
HOMA-IR	1.88 ± 0.09	1.54 ± 0.15	5.67 ± 0.40*	6.57 ± 1.13*	2.81 ± 0.58$^{+,\#,@}$
Serum TG (mg/dl)	77.4 ± 4.9	80.8 ± 5.2	100.2 ± 9.5*	108.6 ± 8.4$^{*,\#}$	87.5 ± 6.3$^{+,\#,@}$
Serum FFAs (mM)	0.16 ± 0.03	0.16 ± 0.02	0.33 ± 0.03*	0.39 ± 0.04$^{*,\#}$	0.22 ± 0.04$^{+,\#,@}$

*: significant as compared to LF group, +: significant as compared to LF-ω-3 group, #: significant as compared to HF-10-week group, @: significant as compared to HF-16-week group at $P \leq 0.05$.

compared by one-way ANOVA followed by *post-hoc* test. Results were considered statistically significant at $P \leq 0.05$. For the glucose tolerance tests, the area under the glucose curve (AUC) was calculated for plasma glucose incremental changes from 0 to 120 min, with a data spreadsheet program Excel (Microsoft, Redmond, WA, USA) using the trapezoidal method and the results were compared between groups.

Results

Effects of HFD and ω-3 Supplementation on Metabolic Markers

At the beginning of the study, the BMI was insignificantly different between groups (Table 1). As expected, at the end of the study, the HFD significantly increased the BMI ($P < 0.05$) in the HF-10- and the HF-16-week groups compared to the LF group and in the HF-ω-3 group compared to the LF- ω-3 group (Table 1). The BMI index was also significantly increased in the HF-16-week group compared to the HF-10-week group. The final BMI in the HF-ω-3 group showed no statistical difference compared to both HF-10-week and HF-16-week groups ($P > 0.05$).

Both the HF-10-week and HF-16-week groups developed glucose intolerance as indicated by their IPGT curve (Fig. 1 A) and the significant high AUC in the IPGTT (Fig. 1 B) compared with those of the LF group ($P < 0.05$). Although the HF-ω-3 group showed a significantly higher AUC compared with the LF-ω-3 group ($P < 0.05$), however, its glucose tolerance was significantly improved compared to that of the HF-16-week group and even compared to that of the HF-10-week group as indicated by the significantly decreased AUC (Fig. 1 B, $P < 0.05$), suggesting that ω-3 can improve the glucose intolerance caused by the HF diet.

Furthermore, the HF group exhibited significantly higher glycemia, insulinemia and HOMA-IR at 10 and 16 weeks of fat feeding compared with the LF group (Table 1, $P < 0.05$). The HF-ω-3 group showed a significantly decreased fasting serum glucose compared to the HF-16-week group, and a significantly improved serum insulin and HOMA-IR compared to similar parameters in both HF groups. Serum insulin and HOMA-IR in the HF-ω-3 group were still significantly increased compared to those of the LF-ω-3 group ($P < 0.05$). These data suggest that ω-3 improves the insulin resistance caused by HF feeding.

Serum TG and FFA (Table 1) showed a similar pattern, with HFD-induced increased levels in the HF and HF-ω-3 groups compared with the LF and LF-ω-3 groups, respectively, as well as in the HF-16-week compared with the HF-10-week group. These parameters were significantly decreased in the HF-ω-3 group compared to those of the HF group both at 10 and 16 weeks of fat feeding ($P < 0.05$).

Serum TNF-α (Fig. 2) was also significantly increased by HFD in the HF and HF-ω-3 groups compared with that of the LF and LF-ω-3 groups, respectively, although serum TNF-α was insignificantly changed in the HF-16-week compared with the HF-10-week group. Serum TNF-α level was significantly decreased in the HF-ω-3 group compared to that of the HF group both at 10 and 16 weeks of fat feeding ($P < 0.05$).

Serum concentration of adiponectin was significantly lowered by HFD in the HF and HF-ω-3 groups compared to that of the LF and LF-ω-3 groups, respectively (Fig. 3, $P < 0.05$). Serum adiponectin was significantly increased in the HF-ω-3 group compared to that of the HF group both at 10 and 16 weeks of fat feeding (Fig. 3, $P < 0.05$), denoting increased adiponectin formation by omega-3 supplementation in the HF fed rats.

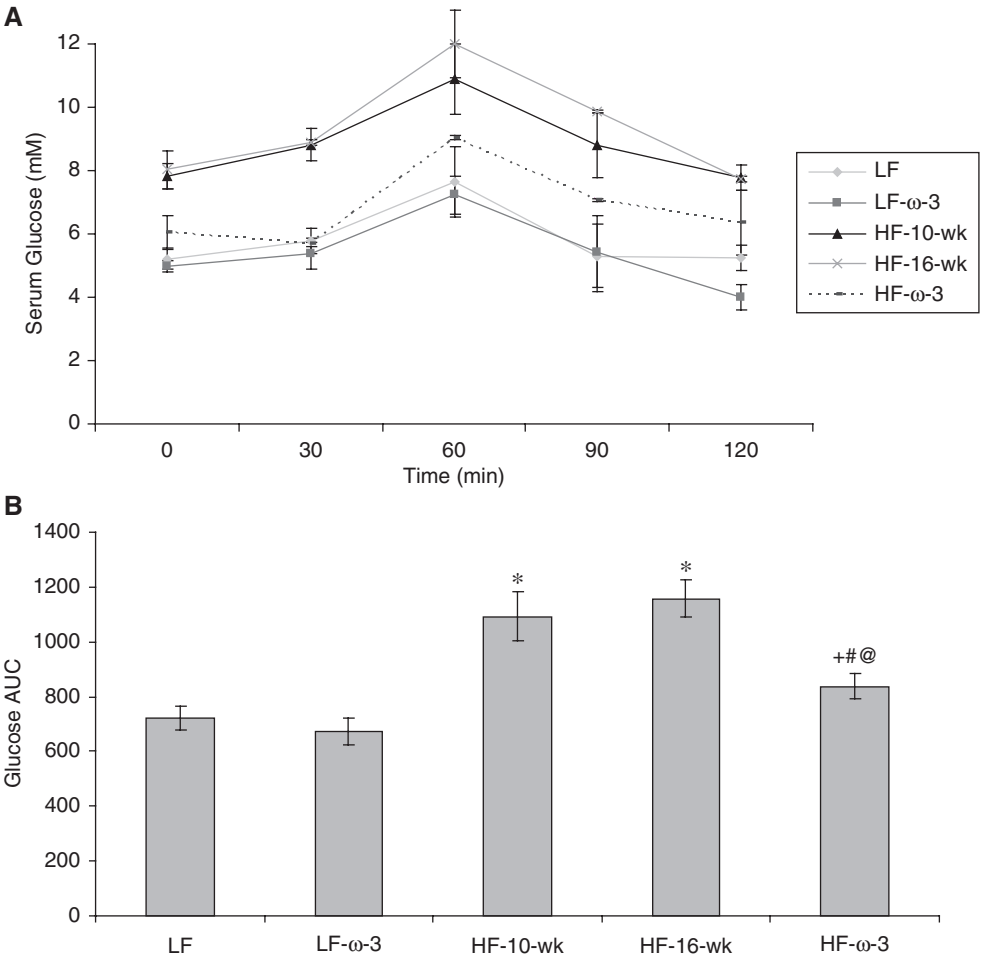

Fig. 1. Glucose time course and area under the curve (AUC) after an intraperitoneal glucose tolerance test (IPGTT) (2 g glucose per kg of body weight). (A) Serum glucose levels during IPGTT in the LF (◆), LF-ω-3 (■), HF-10-week (▲), HF-16-week (✕) and HF-ω-3 (. . .) groups. (B) Glucose AUC: *: significant as compared to LF group, +: significant as compared to LF-ω-3 group, #: significant as compared to HF-10-week group, @: significant as compared to HF-16-week group at $P \leq 0.05$. Values are expressed as means ± SD (n = 7 per group).

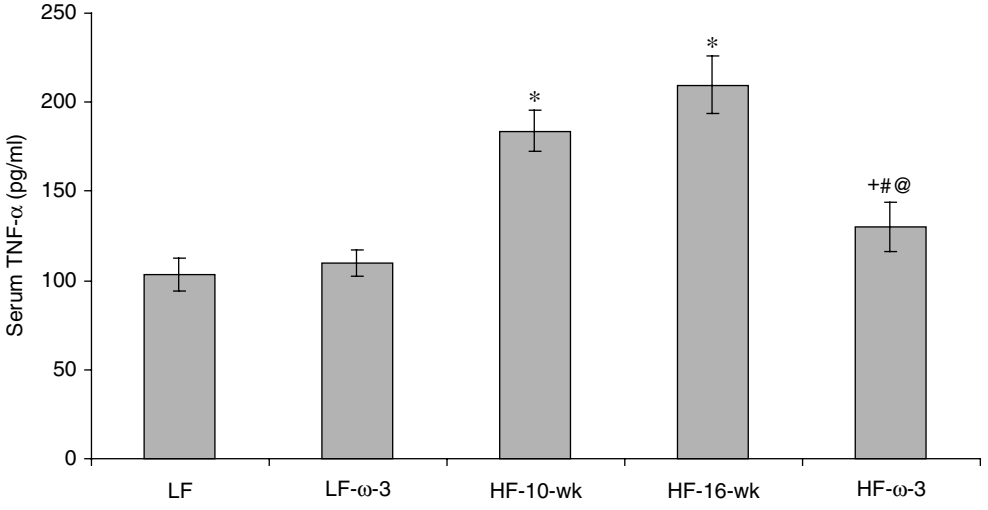

Fig. 2. Effects of HFD and ω-3 supplementation on serum TNF-α. Values are presented as means ± SD (n = 7). *: significant as compared to LF group, +: significant as compared to LF-ω-3 group, #: significant as compared to HF-10-week group, @: significant as compared to HF-16-week group at $P \leq 0.05$.

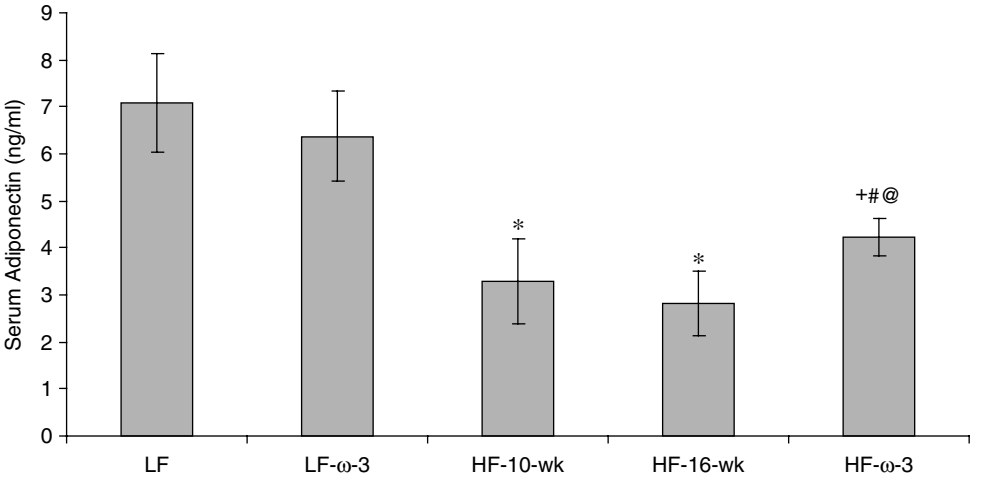

Fig. 3. Effects of high-fat diet and ω-3 supplementation on serum adiponectin. Values are presented as means ± SD (n = 7 per group). *: significant as compared to LF group, +: significant as compared to LF-ω-3 group, #: significant as compared to HF-10-week group, @: significant as compared to HF-16-week group at $P \leq 0.05$.

Table 2. Effects of high-fat diet and omega-3 supplementation on left ventricular dimensions and function assessed by echocardiography

	LF	LF-ω-3	HF-10-wk	HF-16-wk	HF-ω-3
LV Posterior Wall (mm)	1.55 ± 0.12	1.50 ± 0.15	1.70 ± 0.13	1.88 ± 0.26*	1.50 ± 0.19$^{@}$
Ejection Fraction (%)	78.57 ± 5.90	81.42 ± 9.36	80.00 ± 7.48	73.71 ± 7.69	86.00 ± 6.92$^{@}$
Fraction Shortening (%)	41.7 ± 3.8	40.0 ± 5.6	39.7 ± 4.6	38.3 ± 4.8	42.1 ± 4.8
LV End-Systolic Volume (mm)	2.24 ± 0.35	2.31 ± 0.20	2.88 ± 0.16*	2.82 ± 0.52*	2.38 ± 0.32$^{\#, @}$
LV End-Diastolic Volume (mm)	3.77 ± 0.41	3.95 ± 0.23	5.00 ± 0.37*	4.54 ± 0.41*	4.18 ± 0.17$^{\#, @}$

*: significant as compared to LF group, #: significant as compared to HF-10-week group, @: significant as compared to HF-16-week group at $P \leq 0.05$.

The LF-ω-3 group showed no statistical significant difference in the metabolic parameters compared to the LF group.

Effects of HFD and ω-3 on LV Dimensions and Function

As observed in Table 2, echocardiography revealed significant increase of LV end- systolic and -diastolic volumes ($P < 0.05$) by HFD in the HF group after 10 and 16 weeks of fat feeding almost to the same extent compared to the LF group, and both parameters were significantly decreased with ω-3 supplementation in the HF-ω-3 group compared to both HF groups. The LV posterior wall was also significantly increased after 16 weeks of fat consumption and it was significantly decreased by ω-3 supplementation in the HF-ω-3 group. Ejection fraction was significantly increased in the HF-ω-3 group compared to that of the HF-16-week group ($P < 0.05$) although the latter did not show statistical difference with the LF group. There was no echocardiographic measured fat diet or ω-3 effect on LV fraction shortening. No statistical difference was observed in the assessed cardiac function between the LF-ω-3 and LF, HF-ω-3 and LF-ω-3 or HF-16- and HF-10-week groups.

Effects of HFD and ω-3 Supplementation on Heart and Adipose Tissue Adiponectin and Adipo R1 and R2 Expression

Heart and adipose tissue *adiponectin* mRNA levels and adiponectin release (Fig. 4, A and B, respectively) as well as the adipo R1 and R2 gene expression (Fig. 5, A and B respectively) were significantly decreased by HFD in the HF group after 10 and 16 weeks of fat feeding compared to the LF group and also in the HF-ω-3 group compared to the LF-ω-3 group ($P < 0.05$). Heart and adipose tissue *adiponectin* mRNA expression levels, adiponectin release and adipo R1 expression showed no statistical difference in the HF-16-week group compared to the HF-10-week group. However, adipo R2 expression

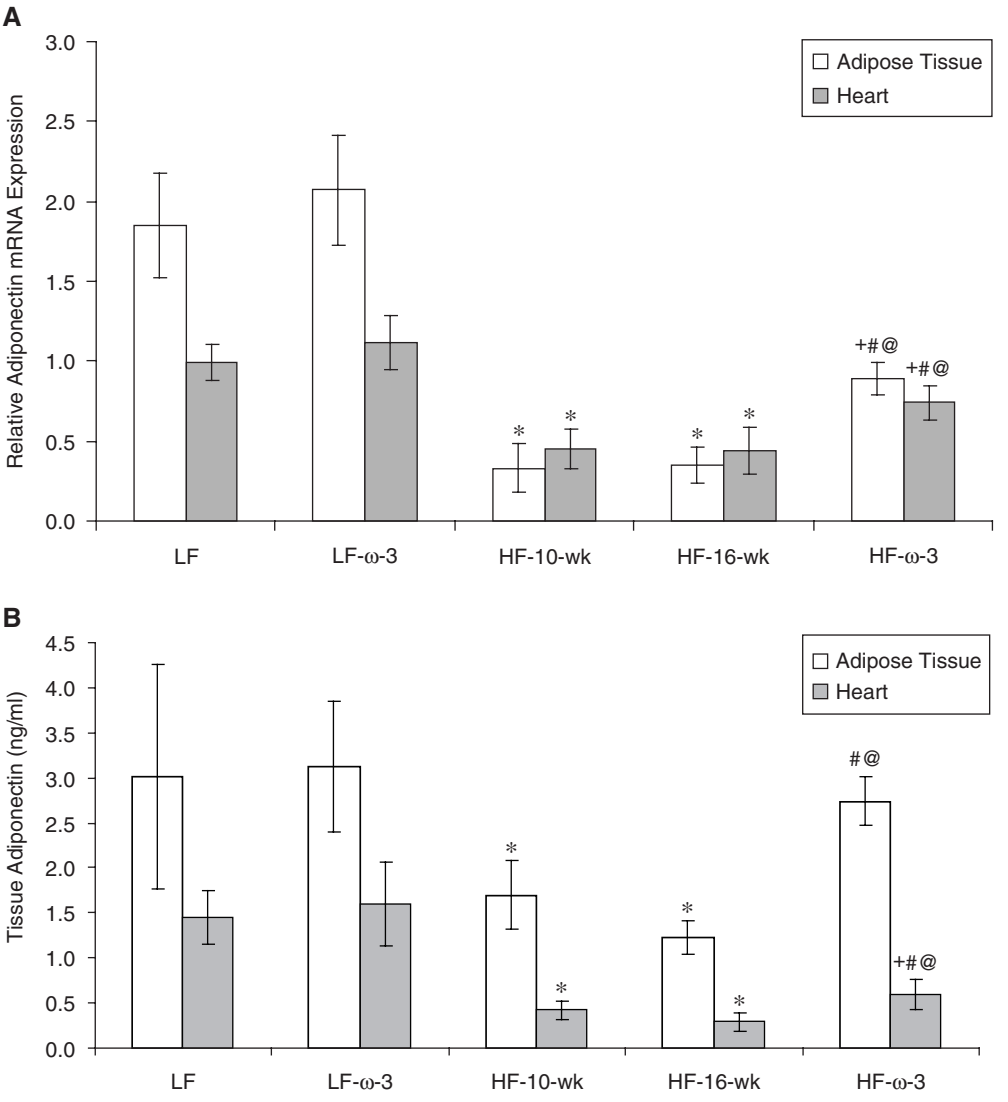

Fig. 4. Effects of HFD and ω-3 supplementation on the heart and adipose tissue adiponectin levels. (A) Total RNA was subjected to quantitative real-time PCR analysis with primers for adiponectin in the adipose tissue (open bars) and in the heart (filled bars) and the mRNA expression levels were normalized to *GAPDH* mRNA levels. (B) Released adiponectin measured by ELISA in homogenates of adipose tissues (open bars) and heart (filled bars). Values are presented as means ± SD (n = 7). *: significant as compared to LF group, +: significant as compared to LF-ω-3 group, #: significant as compared to HF-10-week group, @: significant as compared to HF-16-week group at $P \leq 0.05$.

in the adipose tissue was significantly lower. Supplementation of the HF group with ω-3 significantly increased *adiponectin* mRNA expression, adiponectin release and adipo R1 compared to both HF groups in the heart and adipose tissue and adipose tissue adipo R2 compared to HF-16-week group only ($P < 0.05$). On the other hand, the heart R2 receptor expression was insignificantly changed in the HF-ω-3 group compared to both the HF groups. No significant difference was observed in *adiponectin* mRNA expression levels, adiponectin release and adipo R1 and R2 expression levels in the LF-ω-3 group compared to those of the LF group.

Effects of HFD and ω-3 on Heart pAMPK

Phosphorylation of AMPK (pAMPK) was significantly decreased by HFD in the HF group after 10 and 16 weeks of fat feeding compared o the LF group and in the HF-ω-3 group, compared to the LF-ω-3 group ($P < 0.05$) (Fig. 6, A and B). No statistical significance was observed in heart pAMPK of LF-ω-3 and HF-16-week groups compared to that of LF and HF-10-week groups, respectively ($P > 0.05$). Supplementation of the HF group with ω-3 significantly increased heart pAMPK in the HF-ω-3 group compared to both HF groups ($P < 0.05$) which indicates

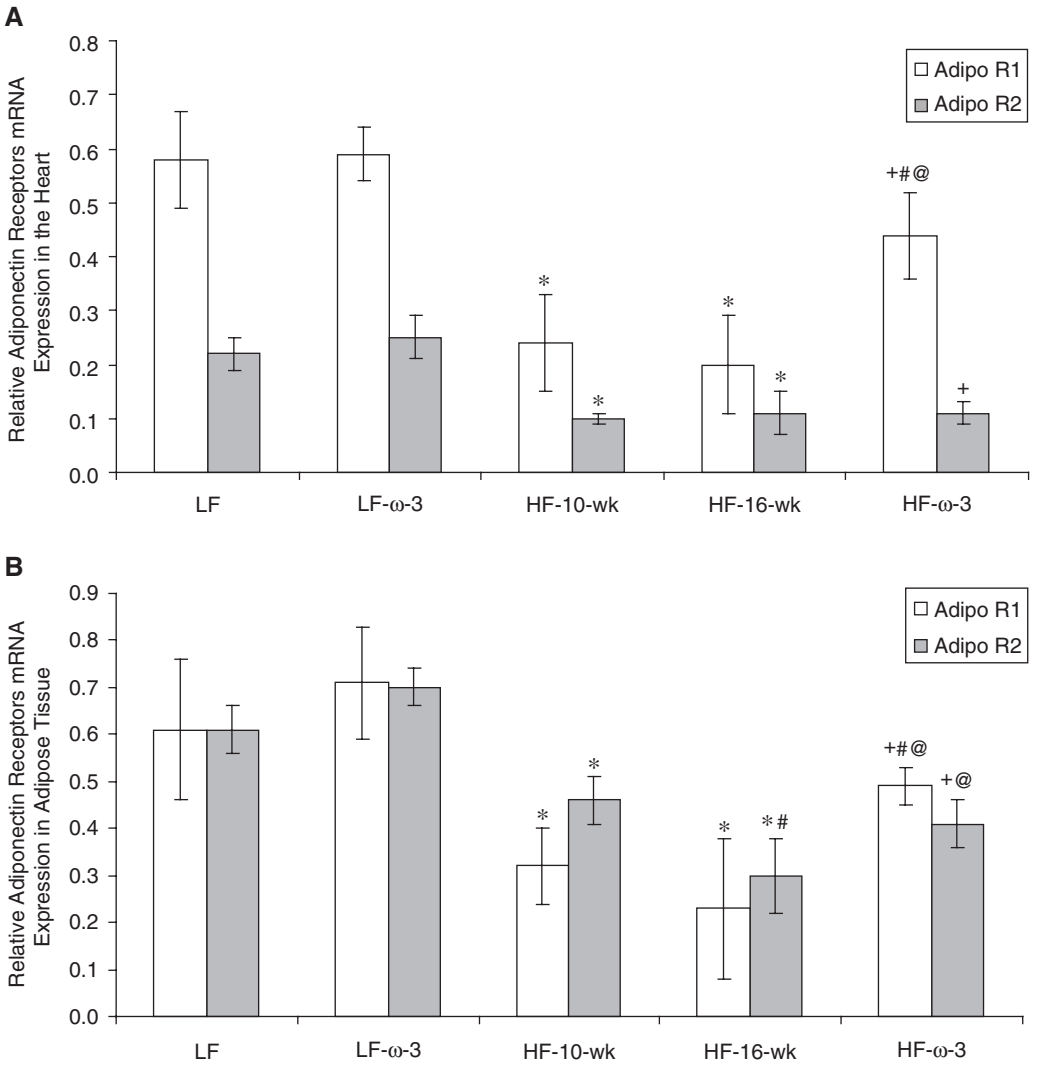

Fig. 5. Effects of HFD and ω-3 supplementation on mRNA expression levels of adiponectin receptors 1 and 2 in the heart and adipose tissue. Total RNA was subjected to quantitative real-time PCR analysis with primers for adipo R1 (open bars) and adipo R2 (filled bars) in heart (A) and adipose tissue (B). mRNA expression levels were normalized to *GAPDH* mRNA expression. Values are presented as means ± SD (n = 7). *: significant as compared to LF group, +: significant as compared to LF-ω-3 group, #: significant as compared to HF-10-week group, @: significant as compared to HF-16-week group at $P \leq 0.05$.

that ω-3 can reverse fat-induced decrease of AMPK phosphorylation in the heart.

Discussion

Adiponectin is thought to play a decisive role in the relationships among obesity, insulin resistance and cardiovascular risk. ω_3 polyunsaturated fatty acid therapy continues to show great promise in the prevention of cardiovascular diseases. In this study, dietary supplementation of the HF group with ω-3 PUFA reversed fat-induced increase in end- diastolic and systolic volumes and left ventricle posterior wall thickness with improvement of the ejection fraction. The beneficial effects of ω-3 PUFA in the HF-ω-3 rats were associated with increased expression of the heart and adipose tissue adiponectin which was decreased by HFD, suggesting that part of the cardio-protective effect of ω-3 depends on the stimulation of adiponectin synthesis.

Similarly, a previous study has found that high-fat feeding is associated with left ventricular contractile dysfunction (6) and a decrease in adiponectin expression in the adipose tissue (1); in the present study, an additional decrease in heart adiponectin expression has been found. It has been postulated that lipids, in particular high levels of triacylglycerols, independent of body mass dysregulate *adiponectin* mRNA expression (27). Neschen *et al*. (31) reported that fish oil activation of adiponectin synthesis in the adipose tissue is regulated by peroxisome proliferator-activated receptor (PPAR) -γ mechanism independent

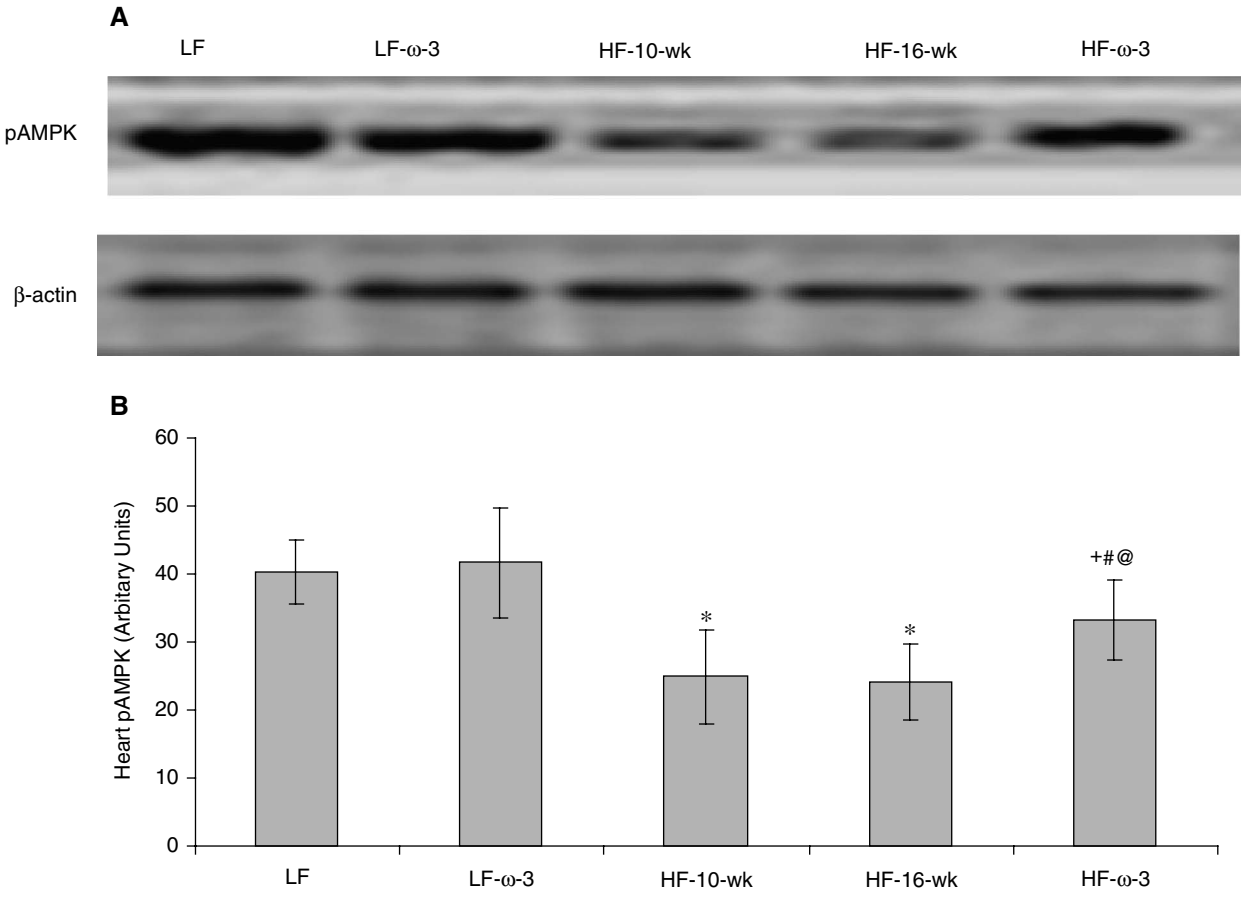

Fig. 6. Effects of HFD and ω-3 supplementation on phosphorylation of AMPK in the heart. (A) Western blot of pAMPK relative to β-actin. (B) pAMPK in the heart of the different groups. Data are presented as mean ± S.D. *: significant as compared to LF group, +: significant as compared to LF-ω-3 group, #: significant as compared to HF-10-week group, @: significant as compared to HF-16-week group at $P \leq 0.05$.

of PPAR-α. Since PPAR-γ is found also in the heart, it can be one of the mechanisms through which ω-3 PUFA increases adiponectin expression in the heart and adipose tissue although PPARs were not measured in this study. In contrast, Shah *et al*. (39) reported that supplementation with fish oil prevented left ventricular hypertrophy in cardiac tissues in response to pressure overload in animals fed the normal low-fat high-carbohydrate diet and that this beneficial effect was not observed with a high saturated fat diet, possibly because of decreased linoleic acid availability and adverse cardiolipin remodeling.

Increased adiponectin expression in the heart of the HF-ω-3 group was associated with a significant increase in phosphorylation of AMPK which was reduced by HFD, suggesting that the cardioprotective effects of ω-3 may occur through adiponectin-induced activation of AMPK signaling. AMPK plays an important role in the regulation of malonyl-CoA content by inhibiting acetyl-CoA carboxylase, leading to an increase in fatty acid oxidation and prevention of fatty acids shift towards formation of complex lipid compounds which are toxic to the heart (23). In contrast, Duda *et al*. (11) observed no changes in AMPK activation in rats subjected to pressure overload after EPA and DHA administration in spite a dose-dependent increase in circulating adiponectin correlated with improved LV function. The authors suggested that the beneficial effects of adiponectin could only be linked to its anti-inflammatory action.

The adipose tissue is the major site of adiponectin secretion. While most of the previous studies have shown that when ω-3 PUFA is combined with HFD before development of obesity can increase plasma adiponectin secondary to reduced adiposity (12, 31), it is unclear if a similar effect occurs after established obesity. In this work, supplementation of the HF group that had been made obese by prior feeding of a HFD for 10 weeks with ω-3 significantly increased adiponectin adipose tissue expression and serum level compared to both 10- and 16-week HF-fed groups despite comparable obesity index. These results are in total agreement with those of Flachs *et al*. (13) and suggest adiposity-independent effects of ω-3 on

adiponectin secretion.

In consistent with our results, a previous research (32) showed a positive effect of EPA or DHA on glucose tolerance and insulin resistance in HF-fed rats. Hyperinsulinemia and cardiac insulin resistance with obesity may lead to cardiac dysfunction and LV hypertrophy, perhaps through insulin activation of cardiac protein synthesis and inhibition of protein breakdown (28, 40). The improvement of serum insulin level and insulin resistance by ω-3 supplementation suggests an additional cardioprotective effect.

The repressive effects of HFD on the heart and adipose tissue adipo R1 and R2 expression observed in this study agree with those previously reported in the adipose tissue, skeletal muscle and liver related to hyperglycemia and insulinemia (9) and with similar results in the heart (36) although these data disagree with data presented by Bullen *et al*. (3). There are inconsistent reports regarding the relationship between receptor expression and insulin. It has been shown that insulin represses receptor mRNA expression in both muscle and liver *via* activation of phosphatidylinositol 3-kinase and inactivation of Foxo1 (43). However, Inukai *et al*. (15) reported that adipo R1 but not adipo R2 expression was repressed after insulin administration. Free fatty acid levels were also found to regulate receptor expression (21). PPAR-γ agonist stimulation was previously found to up-regulate the cardiac adipo R1 expression without affecting the expression of adipo R2 in high fat-fed rats (36). In this study, however, ω-3 supplementation was found to increase adipo R1 expression in the heart and adipo R1 and R2 receptors expression in the adipose tissue. It is possible that the ω-3-induced improvements in glucose, insulin and lipid profiles had led to the differences observed in the receptor expression.

Given that obesity is defined as a state of low-grade inflammation (42) characterized by over expression of TNF-α (4), we also measured serum TNF-α which was found to be significantly increased by HFD and significantly reduced by ω-3 supplementation, consistent with previous studies (26, 29). Because TNF-α has been linked with insulin resistance (7), cardiac contractile dysfunction (25) and suppression of heart adipo R1 expression (38), ω-3 can have additional beneficial cardioprotective effects by decreasing TNF-α. Possible mechanisms include activation of AMPK (20) and modulation of transcription factors such as the nuclear transcription factor kappa B (NF-κB) (44) known to be also modulated *via* adiponectin (34) and PPARs-γ (16).

Some of the cardioprotective effects of ω-3 fatty acids could also result from their favorable effects on the lipid profile. Supplementation of the HF group with ω-3 decreased both serum TG and free fatty acids induced by HFD. The hyperlipidemia that frequently accompanies obesity may increase cardiac lipid uptake and formation of ceramide and other toxic lipids, resulting in oxygen wasting and lower cardiac mechanical efficiency (28). EPA and DHA are able to reduce hepatic *de novo* fatty acid synthesis and to increase both PPAR-α and mitochondrial fatty acid β-oxidation (18).

In conclusion ω-3 can induce improvements on cardiac dysfunction produced by HFD through its up-regulating effects on both adiponectin and adiponectin receptors expression in the heart and adipose tissue, and improvement of insulin resistance in addition to its anti-inflammatory and hypolipidemic actions.

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