Acta Biomed 2013; 84: 53-60

Original article

A sturgeon-derived bioactive compound beneficially modulates nuclear receptors controlling metabolic functions in patients with metabolic syndrome

Aldo Lorenzetti[1], Roberto Catanzaro[2], Chiara Sapienza[1], Nicola Zerbinati[3], Shalini Jain[4], Umberto Solimene[5], Santos K. Yaduvanshi[6], Nalini Srivastava[6], Michele Milazzo[2], Hariom Yadav[4], Angelo Italia[2], Francesco Marotta[1]

[1] ReGenera Research Group for Aging-Intervention, Milano, Italy; [2] Dept of Internal Medicine, University of Catania, Catania, Italy; [3] CMP-Medical Center, Pavia, Italy; [4] National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, USA; [5] WHO-cntr for Traditional Medicine & Biotechnology, University of Milano, Italy; [6] School of Studies in Biochemistry, Jiwaji University, Gwalior, India

Abstract. The aim of the present study was to test the possible effects of a novel sturgeon-derived compound (LD-1227) on inflammatory markers related to metabolic nuclear receptors in patients with metabolic syndrome. The study population consisted of 76 patients with metabolic syndrome and 30 healthy subjects who were maintained to their current treatments and randomly supplemented: A) LD-1227 (n=38) or B) placebo (n=38) as compared to C) healthy controls (n=30). LD-1227 or placebo (water-soluble starch) were given daily at breakfast and dinner for three months. Levels of hs-CRP, IL-6, TNF-α, leptin and adiponectin/resistin index were assayed at the entry, 1 month and 3 months afterwards. At the end of the study period, as compared to B group, LD-1227-treated patients showed a significant improvement of all parameters tested, irrespective of the presence of diabetes. In particular, levels of adiponectin and adiponectin/resistin index significantly increased following LD-1227 administration. Although the metabolic syndrome remains a multifaceted condition requiring a complex approach, LD-1227 could be a potential safe therapeutic tool to be integrated into a wider treatment and preventive medicine schedule strategy. (www.actabiomedica.it)

Key words: marine collagen peptide, peroxisome proliferator-activated receptor (PPAR), liver X receptor, farnesoid X receptor, metabolic nuclear receptors, LD-1227, metabolic syndrome

Introduction

Although not all overweight individuals develop insulin resistance and its associated metabolic abnormalities, all evidences clarify that the increasing prevalence of overweight and obesity is a fundamental contributor to the rising prevalence of the metabolic syndrome (MS) and type 2 diabetes. The MS represents nowadays a common illness and is associated with higher incidence of type II diabetes mellitus and cardiovascular mortality while the several classifications share a mutual background. In fact, different groupings of following metabolic factors are required in each classification of the MS, including: insulin resistance, low HDL-C, hypertriglyceridemia, obesity or increased waist girth, impaired glucose tolerance or diabetes mellitus, microalbuminuria, hyperinsulinemia and hypertension. In the present study we used the definition of MS as proposed by the American Heart Association and the National Heart, Lung, and Blood Institute (1). These criteria require at least three of the following components: abdominal obesity, triglycerides

≥150 mg/dl or under drug treatment, HDL cholesterol <50 mg/dl or receiving drug treatment, systolic/diastolic blood pressure ≥130/85 mmHg or receiving drug treatment, and fasting glucose ≥100 mg/dl or receiving drug treatment. Patients with MS have at least a 2-fold increase in risk for ASCVD, as compared to those without (1). However, the relationship between metabolic risk factors and development of ASCVD is still a matter of investigations. Since the MS is casually linked to obesity-related insulin resistance, the detection of peroxisome proliferator-activated receptor gamma (PPARγ) as a key regulator of adipogenesis and insulin resistance (2) has represented an essential prove of concept. Indeed, PPARγ targets a multitude of downstream genes in adipocytes and is the only known factor that is necessary and sufficient for induction of adipocyte differentiation (3,4). As a class of ligand-activated transcriptional factors including PPARs, LXRs, RXR and FXRs, metabolic nuclear receptors have thus attracted an increasing attention from the medical community for their role in regulating lipid and glucose homeostasis. A number of specific synthetic agonists or modulators of nuclear receptors have been tested and are under study and therapeutic agents designed to affect pathways involving metabolic nuclear receptors may be beneficial for subjects prone to develop and patients with MS.

In this regard marine organisms represent a huge repository of useful bioactive compounds and an increasing scientific evidence demonstrates that marine biology molecules may have a wide spectrum of effects, such as anti-inflammatory (5), tumor suppression, antihypertensive, by inhibiting angiotensin I converting enzyme activity (6) and anti-dyslipidemia (7).

Overall, the roles marine n-3 poly-unsaturated fatty acids (PUFAs) in regulating inflammatory processes and responses indicates that the level of exposure to these fatty acids might be relevant in determining the onset and degree of severity of inflammatory diseases. PUFAs can be incorporated into the phospholipids of inflammatory cell membranes where they act modulating the environment for membrane protein functional response, maintaining membrane fluidity and influencing lipid raft development (8). Membrane phospholipids represent substrates for the generation of second messengers and can modify their activity (9). In addition, membrane phospholipids are substrates for the release of (non-esterified) PUFAs intracellularly – the released PUFAs can act as signaling molecules, ligands for transcription factors, or precursors for biosynthesis of lipid mediators which are involved in regulation of many cell and tissue responses, including inflammation and immunity. The above data have suggested that dietary supplementation with marine n-3 PUFAs of patients with inflammatory diseases may bring about a clinical advantage. However, the multitude of potential mechanisms involved and the diversity of experimental designs often using concentrations clinically inapplicable make it difficult to fully understand the actions of marine compounds within disease processes. Nonetheless, there are increasing evidences of their efficacy such as, for example, that treatment with dietary cod proteins may improve insulin sensitivity in insulin-resistant individuals and reduced insulin-resistance related metabolic disorders (10). Our recent studies have highlighted the effect of a biomarine compound (a bacteriologically-free, sturgeon-egg derived protein gel preparation, 400 mg marine protein/capsule, which has a protein/lipid ratio of 3.6 and the following major class of fatty acids: saturated fatty acids: 23%, monounsaturated fatty acids 33%, polyunsaturated fatty acids 34%, with a median n-6/n-3 fatty acid ratio of 2.7. In particular, the main fatty acids g/100g of total fatty acids are as follows: C22:6 5.8, C16:0 15.8, C18:1 33.7 and C18:2 24.4. LD-1227, Caviarlieri, D & S Group, Singapore) on MMPs, collagen metabolism and on chrondrocyte inflammatory markers (11, 12). Thus, the aim of the present work was to test whether such bioactive compound may beneficially modulate the ongoing inflammation and the pathways involving metabolic nuclear receptors in metabolic syndrome such as PPARs, LXRs and FXRs in patients with MS.

Material and methods

Subjects

Informations collected in baseline evaluations included data regarding demographic characteristics, personal and family medical history, alcohol consump-

tion, smoking history, number of deliveries, oral contraceptive use, hormone replacement therapy, breast feeding and age at menarche/menopause. Patients with secondary hypertension, cardiomyopathy, severe abnormalities of liver and kidney function, cerebrovascular diseases, grossly elevated total cholesterol (>280mg/dl or LDL >180mg/dl), malignancies and history of coronary bypass surgery or on insulin treatment were excluded from this study. Healthy controls had no past or present history of any relevant disease and had no abnormality of the main biochemical findings. The study population consisted 76 patients with metabolic syndrome and 30 age-matched healthy volunteers. All subjects participated in the study on a voluntary and informed basis. Written consent was obtained from all subjects before the study and the study conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

S*tudy Design*

All MS patients were kept on their usually prescribed oral hypoglycaemics and/or anti-hypertensive drugs) but without anti-hyperlipidemic medications. All anthropometric measurements were taken by the same well-trained nurse. Weight was measured to the nearest 0.1 kg using a calibrated digital scale with subjects standing barefoot in light clothing. Height was measured to the nearest 1 mm with a portable stadiometer (Seca Ltd, Hamburg, Germany), with the subject upright and the head in the Frankfurt plane. Waist circumference was measured to the nearest 1 mm with a flexible, non-elastic tape midway between the tenth rib and the iliac crest at the end of a gentle expiration. Body Mass Index (BMI) was calculated as follows: weight (kg)/height squared (m^2). All subjects were divided into 3 groups: LD-1227-treated patients (*n*=38, group A), placebo-treated patients (*n*=38, group B) and healthy controls (*n*=30). The two patients groups were matched as for gender, duration of established diagnosis and variety of pathological conditions underlying the metabolic syndrome classification (table 1). Supplements and placebo were given at breakfast and dinner. Brachial artery blood pressure was measured with a sphygmomanometer with the subject in a sitting position at rest for two minutes. No specific change on diet and lifestyle was prescribed to all subjects although it was made sure that none of them had grossly irregular attitudes. LD-1227 or placebo (water-soluble starch) were taken daily before breakfast and bedtime at a dose of 1cp (total 2 cp/day) in parallel with routine clinic drugs for three months. Subjects were randomized using a table of random numbers derived from a random number generating program. A single master sheet was used to randomize subjects if they were eligible for inclusion in the study following the clinical screen. The master sheet included subject number, coordinator initials and date of randomization was used to track the process and insure no duplications of assignment.

Table 1.

Parameters	Healthy Control	Untreated MS	MS patients treated with LD-1227
Total n. (male/female)	30 (15/15)	38 (20/18)	38 (18/20)
Mean age	67	71	66
Mean BMI (range)	23 (21-25)	27 (22-36)	26 (24-33)
Family history of diabetes	4/30	14/38	21/38
Family history of hypertension	2/30	9/38	13/38
Overt diabetes	ND	11/38	16/38
Duration of diabetes (years)	ND	3	5
Dyslipidemia	1/30	12/38	18/38
Smoking	4/30	14/38	11/38
Waist (cm)	82	98	99
Waist/hip ratio	0.82	0.93	0.96
Mean Systolic Blood Pressure (mmHg)	124	157	165
Mean Diastolic Blood Pressure (mmHg)	71	88	86
Physical activity	8/30	4/38	2/38

Blood tests

Blood was collected in the morning (between 7 and 9 A.M.) via venipuncture in a supine resting position after an overnight fast. Serum was isolated and refrigerated overnight in plastic tubes, at which time aliquots were prepared and stored at-80°C. The serum concentrations of IL-6, hs-CRP, TNF-α, leptin (all with a CV of < 4.6%), resistin and adiponectin (CV of 3.5%) before intervention, 1 months and 3 months after intervention. were determined by using Quantikine high-sensitivity immunoassay kits (R&D Systems, Minneapolis, MN, USA). All samples were measured in duplicate and the average of the 2 values was used for final data analysis. The sensitivity of these assays was 0.018 pg/ml for IL6, 0.1 mg/l for hsCRP and 0.039 pg/ml for TNF-α. The intra- and inter-assay coefficients of variation were <10% for both cytokines. Duplicate samples that did not provide a coefficient of variation < 10% were re-analyzed and all values were averaged for data analysis. Samples with undetectable concentrations were assigned a value corresponding to the lower limit of detection of the assay (2 pg/ml for IL-6 (27%), 4.0 pg/ml (13%) for TNFα and 0.01 mg/l (22%) for hs-CRP). After a further review of the data for outliers, all analyses were carried out excluding participants with hsCRP levels >10 mg/l or those with missing data on more than 2 biochemical parameters (*n*=3). This decision altered only the point estimates, but the direction of the effect did not vary from the analysis of the whole sample.

Metabolic Nuclear Factors function

Markers of metabolic nuclear receptors including leptin, resistin and adiponectin were assayed by enzyme-linked immunoassay using reagents from Otsuka Pharmaceuticals, Tokyo. Resistin is a macrophage-derived signalling polypeptide hormone which has been shown to be up-regulated in subjects with diabetes, MS and cardiovascular disease (13). Adiponectin is an adipocyte-secreted polypeptide hormone which regulates insulin sensitivity and energy homeostasis, as well as glucose and lipid metabolism (14) Adiponectin exerts an insulin-sensitizing action via an enhancement of AMPK and PPARα, this having profound effects on fatty acid oxidation and inflammation. As a index highly related to metabolic syndrome, Adiponectin-resistin (AR) index was calculated as recently described (15), in a multiplicative inverse relation, as follows: $\alpha=(1/A_0)xR_0=R_0/A_0$. Then, the resulting value is logarithmically transformed for normalization as follows: $\beta=\log_{10}(\alpha)=\log_{10}(R_0/A_0) = \log_{10}(R_0)-\log_{10}(A_0)$. Finally, a numerical constant 1 is added to the latter resulting value to get a positive integer of the AR index which is expressed as such: $1 + \log_{10}(R_0)-\log_{10}(A_0)$ where R_0 and A_0 indicate fasting serum total resistin levels expressed in ng/mL and fasting serum total adiponectin levels expressed in g/mL, respectively.

Statistical Analysis

Statistical analysis was performed using the x2 statistic or Fisher's Exact Test for independence. Pairwise analysis was performed as appropriate. Two-tailed tests of significance were used throughout. Correlations were assessed using linear regression analysis. When necessary, log transformation was used to normalize the data, or appropriate nonparametric tests were employed. If factor time was significant, post hoc tests with Bonferroni correction were carried out to compare concentrations to baseline concentrations. Data are presented as the median (with the range in parentheses) given the apparent departure of data from distributional normality. The required significance level for all tests was set at $p<0.05$.

Results

No side effects or changes of systolic and diastolic blood pressure or anthropometric parameters were observed throughout the study nor any gastrointestinal complaints or unfavourable organolectic report..

Effect of LD-1227 supplementation on serum IL-6, TNF-α and hs-CRP Levels

Throughout the whole study, the level of IL-6 and TNF-α was significantly higher in MS patients as compared to healthy controls and it was not affected by the presence of diabetes ($p<0.05$, fig 1). After in-

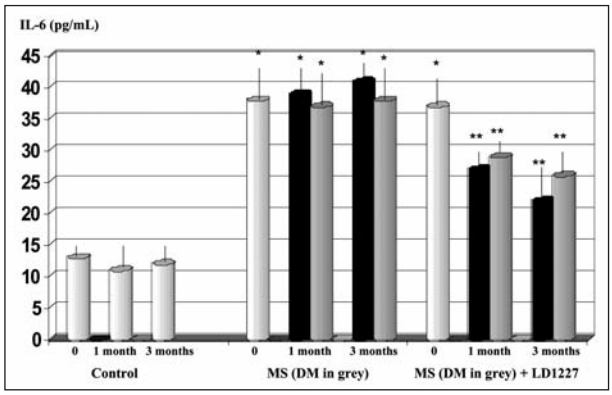

Figure 1a. Time course change of IL-6 In patients with ms with/without diabetes: effect of treatment with LD-1227
Legend: different colours indicate different observation time, as depicted on the top of the figure. See text for abbreviations. *p<0.001 vs healthy controls; **p<0.01 vs untreated MS.

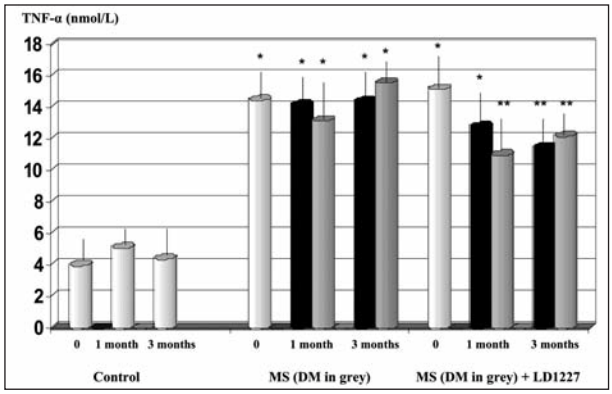

Figure 1b. Time course change of TNFα in patients with ms with/without diabetes: effect of treatment with LD-1227
Legend: different colours indicate different observation time, as depicted on the top of the figure. See text for abbreviations. *p<0.001 vs healthy controls; **p<0.01 vs untreated MS

tervention with LD-1227, levels of both cytokines decreased significantly in all MS patients (p<0.05 vs baseline and vs placebo group). Moreover, the level of hs-CRP in patients with MS was significantly higher than in healthy controls (p<0.05, fig 2). and particularly in those with overt diabetes (p<0.05 vs MS patients without diabetes) and significantly decreased at a comparable extent after LD-1227 intervention, irrespective of diabetes (p<0.05 vs placebo). The presence of hypertension didn't affect the significance of the data, as by a multivariate analysis while patients with

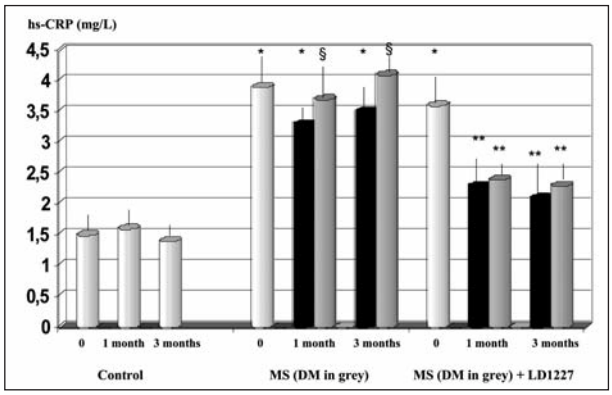

Figure 2. Time course change of hs-CRP In patients with ms with/without diabetes: effect of treatment with LD-1227
Legend: different colours indicate different observation time, as depicted on the top of the figure. See text for abbreviations. *p<0.001 vs healthy controls; §p<0.05 vs MS patients without DM; **p<0.01 vs untreated MS

overt obesity (BMI over 30) showed at baseline a not significant trend increase of IL-6 (data not shown).

Effect of LD-1227 supplementation on leptin and adiponectin/resistin index

As compared to healthy control, the levels resistin were all significantly higher at each observation check in MS patients and higher values were recorded in patients with overt obesity (BMI over 30, data not shown). However, the limited number of this subgroup didn't allow a statistical significance. On the other hand, adiponection showed only a marginal non significant decrease in our MS patients. Overall, the adiponectin/resistin (AR) Index was significantly higher in all MS patients (p<0.001 vs control, figure 3) and, namely in obese diabetic patients (p<0.05 vs untreated MS patients without overt diabetets and diabetes). Moreover, following LD-1227 intervention, AR index showed a time-course decrease which reached a statistical difference at 3 month-observation (p<0.05 vs untreated MS patients).

Discussion

MS represents a cluster of metabolic abnormalities including abdominal obesity, hypertension, hyper-

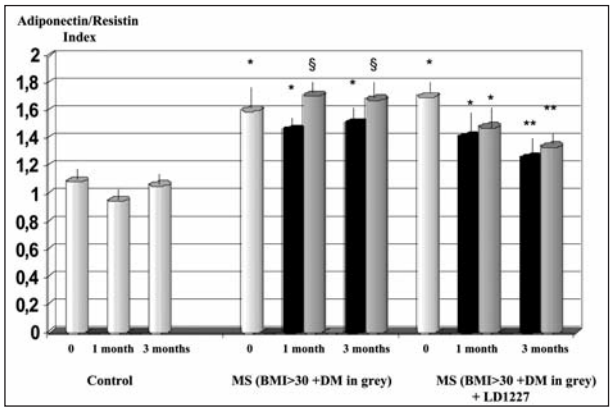

Figure 3. Time course change of ar index in patients with ms with/without overt obesity and diabetes: effect of treatment with LD-1227
Legend: different colours indicate different observation time, as depicted on the top of the figure. See text for abbreviations. *p<0.001 vs healthy controls; §p<0.05 vs MS patients without overt obesity and diabetes; **p<0.05 vs untreated MS.

lipidemia, and diabetes. This condition confers an increased risk for vascular disease-associated morbidity and mortality as well as all-cause mortality, even in the absence of clinically evident cardiovascular disease (CVD) and/or diabetes mellitus (DM) (16). The nuclear receptor (NR) family of transcription factors (also referred to as the steroid/thyroid hormone receptor superfamily) is quite large with approximately 150 proteins. This large superfamily may be categorized into three subgroups: classic hormone receptors such as the glucocorticoid, estrogen, retinoic acid, thyroid hormone, and vitamin D receptors; orphan receptors such as apolipoprotein A-I regulatory protein-1 (ARP-1) and chicken ovalbumin upstream promoter transcription factor and "sensor" receptors such as the peroxisome proliferator-activated receptors (PPARs), liver X receptor (LXR), farnesol X receptor (FXR) the retinoid X receptor (RXR). As a class of ligand-activated nuclear receptors closely involved in metabolism, PPARs, LXRs, and FXRs have been attracting increasing attention in the study of pathogenesis and treatment of metabolic diseases. A growing body of evidence has shown that they exert a crucial role in controlling lipid and glucose homeostasis (17-19). For instance, PPARγ, a member of the nuclear receptor superfamily, is a ligand-regulated transcription factor that is an essential regulator of adipogenesis and insulin resistance in adipose tissue, and, in particular, PPARγ2 expression is restricted to mature adipocytes and this isoform is induced earlier and more strongly than PPARγ1 during adipogenesis (20). Thus, this receptor is implicated in the MS, colon cancer and other chronic diseases and MS outcome has been found to be associated with PPARγ gene polymorphism or mutation (21). It forms a heterodimer with RXRs and binds to a specific DR-1 motif (direct hexa-nucleotide repeats, separated by a single nucleotide) of PPAR response elements. In the present study, we examined effects of LD-1227 on markers of PPARs, RXR, LXRs, and FXRs in MS patients with/without overt diabetes. Results from our study showed that LD-1227 could beneficially affect levels of a wide range of molecules involved in this disorder. Marine compounds continue to provide worthwhile opportunities of clinically-applicable biomolecules. For instance, it has been found that small collagen peptides in the hydrolysate of livestock and fish as well as catfish protein isolate may inhibit the activity of angiotensin I converting enzyme (22) and reducing blood pressure (23). Previous studies have established the correlation of hs-CRP with diabetes (24). Consistent with these findings, in the present study we demonstrated that levels of hs-CRP and IL-6 were significantly higher in MS patients. Indeed, IL6 is considered an important inducer of the hepatic secretion of CRP and plasma levels of IL6 and CRP have been reported to be good predictors of type 2 diabetes in humans (25). However, after LD-1227 administration, levels of all tested cytokines in MS patients markedly decreased whereas their levels remained unchanged in patients receiving placebo. This suggests that LD-1227 could provide protection for MS patients and that this protection was proportionally more evident for patients with diabetes. To further assess effects of LD-1227 on MS patients, we performed assays on changes of three relevant adipocyte-secreted hormones: leptin, resistin and adiponectin, which are also highly implicated in diabetes (26). Indeed, Havel et al. (27)] has shown that adiponectin levels in patients with type 2 diabetes were lower than in non-diabetic patients. A study demonstrated that adiponectin could increase insulin sensitivity and acts with leptin to normalize insulin

action in severely insulin-resistant animals. Indeed, Steppan et al. (28) proved that administration of anti-resistin antibody improved blood glucose and insulin action in mice with diet-induced obesity and suggested that resistin potentially could act as a bridge between obesity and diabetes. Following LD-1227 intervention, levels of leptin and resistin tended to significantly decrease, whereas levels of adiponectin showed a concomitant increase which reached a statistically significant level in obese subjects. More remarkably, the intervention with LD-1227 proved to significantly enhance the adiponectin/resistin (AR) index. This holds particular important when considering that, confirming earlier reports of a significant inverse correlation between these two parameters (29, 30), the AR index has been very recently found to be more strongly associated with increased risk of type 2 diabetes and metabolic syndrome than adiponectin and resistin levels alone (15).

In a most recent work, we have shown in human chondrocytes that this sturgeon-derived compound could effectively inhibit IL-1β-induced proliferation and inflammatory reactions via inhibited activation of the transcription factor NF-κB pathway (8). Indeed, this compound contains collagen elastin, protein and a rich array of other smaller unsaturated fatty acids, and structural phospholipids to be fully defined as yet which may exert a synergistic action on multiple mechanisms of the inflammatory cascade.

We purposely didn't address the issue of lipid profile because this would have required a more defined selection of patients and dietary assessment control. However, preliminary data were not consistent.

Taken together, results from this study showed the beneficial effects of LD-1227 against some fundamental mechanisms affecting the levels of molecules involved in MS. While drugs such synthetic PPAR agonists are under constant development for treating dyslipidaemia, insulin resistance and overt diabetes, the safe profile and advantageous effect of LD-1227 on the regulation of key metabolic nuclear receptors functions make it an amenable tool for long-term maintenance treatments. Moreover, it can represent a potential weapon to be integrated within more articulated preventive medicine regimens in healthy subjects prone to the disease.

References

1. Grundy SM, Hansen B, Smith SC Jr, Cleeman JI, Kahn RA; American Heart Association; National Heart, Lung, and Blood Institute; American Diabetes Association Clinical management of metabolic syndrome: report of the American Heart Association/National Heart, Lung, and Blood Institute/American Diabetes Association conference on scientific issues related to management. *Circulation* 2004; 109: 551-6.
2. Tontonoz P, Hu E, Devine J, Beale EG, Spiegelman BM. PPAR gamma 2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. *Mol Cell Biol* 1995; 15: 351-7.
3. Barak Y, Nelson MC, Ong ES, et al. PPAR gamma is required for placental, cardiac, and adipose tissue development. *Mol Cell* 1999; 4: 585-95.
4. Rosen ED, Spiegelman BM. Molecular regulation of adipogenesis. *Annu Rev Cell Dev Biol* 2000; 16: 145-71.
5. Gillies PJ, Bhatia SK, Belcher LA, Hannon DB, Thompson JT, Vanden Heuvel JP. Regulation of inflammatory and lipid metabolism genes by eicosapentaenoic acid-rich oil. *J Lipid Res* 2012; 53: 1679-89.
6. Cao W, Zhang C, Ji H, Hao J. Optimization of peptic hydrolysis parameters for the production of angiotensin I-converting enzyme inhibitory hydrolysate from Acetes chinensis through Plackett-Burman and response surface methodological approaches. *J Sci Food Agric* 2012; 92: 42-8.
7. Aneiros A, Garateix A Bioactive peptides from marine sources: pharmacological properties and isolation procedures. *J Chromatogr B Analyt Technol Biomed Life Sci* 2004; 803: 41-53.
8. Aliche-Djoudi F, Podechard N, Chevanne M, et al. Physical and chemical modulation of lipid rafts by a dietary n-3 polyunsaturated fatty acid increases ethanol-induced oxidative stress. *Free Radic Biol Med* 2011; 51: 2018-30.
9. Elvers M, Grenegård M, Khoshjabinzadeh H, et al. A novel role for phospholipase D as an endogenous negative regulator of platelet sensitivity. *Cell Signal* 2012; 24: 1743-52.
10. Pilon G, Ruzzin J, Rioux LE, et al. Differential effects of various fish proteins in altering body weight, adiposity, inflammatory status, and insulin sensitivity in high-fat-fed rats. *Metabolism* 2011; 60: 1122-30.
11. Marotta F, Polimeni A, Solimene U, et al. Beneficial modulation from a high-purity caviar-derived homogenate on chronological skin aging. *Rejuvenation Res* 2012; 15: 174-7.
12. R Catanzaro, F Marotta, S Jain, et al. Beneficial effect of a sturgeon-based bioactive compound on gene expression of tumor necrosis factor-α, matrix metalloproteinases and type-10 collagen in human chondrocytes. *J Biol Reg Homeost Agents* 2012; 26: 189-97.
13. Singh AK, Tiwari S, Gupta A, Natu SM, Mittal B, Pant AB. Association of resistin with metabolic syndrome in Indian subjects. *Metab Syndr Relat Disord* 2012; 10: 286-91.
14. Al-Attas OS, Al-Daghri NM, Alokail MS, et al. Association of body mass index, sagittal abdominal diameter and

waist-hip ratio with cardiometabolic risk factors and adipocytokines in Arab children and adolescents. *BMC Pediatr* 2012; 12: 119.
15. Lau CH, Muniandy S. Novel adiponectin-resistin (AR) and insulin resistance (IRAR) indexes are useful integrated diagnostic biomarkers for insulin resistance, type 2 diabetes and metabolic syndrome: a case control study. *Cardiovasc Diabetol* 2011; 21: 10-8.
16. Wu SH, Liu Z, Ho SC. Metabolic syndrome and all-cause mortality: a meta-analysis of prospective cohort studies. *Eur J Epidemiol* 2010; 25: 375-84.
17. Laffitte BA, Chao LC, Li J. Activation of liver X receptor improves glucose tolerance through coordinate regulation of glucose metabolism in liver and adipose tissue. *Proc Natl Acad Sci USA* 2003; 100: 5419-24.
18. Duran-Sandoval D, Mautino G, Martin G. Glucose regulates the expression of the farnesoid X receptor in liver. *Diabetes* 2004; 53: 890-8.
19. Ahmed W, Ziouzenkova O, Brown J, et al. PPARs and their metabolic modulation: new mechanisms for transcriptional regulation? *J Intern Med* 2007; 262: 184-98.
20. Liberato MV, Nascimento AS, Ayers SD, et al. Medium chain fatty acids are selective peroxisome proliferator activated receptor (PPAR) γ activators and pan-PPAR partial agonists. *PLoS One* 2012; 7(5): e36297. Epub 2012 May 23.
21. Capaccio D, Ciccodicola A, Sabatino L, et al. A novel germline mutation in peroxisome proliferator-activated receptor gamma gene associated with large intestine polyp formation and dyslipidemia. *Biochim Biophys Acta* 2010; 1802: 572-81.
22. Theodore AE, Kristinsson HG. Angiotensin converting enzyme inhibition of fish protein hydrolysates prepared from alkaline-aided channel catfish protein isolate. *J Sci Food Agric* 2007; 87: 2353-7.
23. Kim SK, Ngo DH, Vo TS. Marine fish-derived bioactive peptides as potential antihypertensive agents. *Adv Food Nutr Res* 2012; 65: 249-60.
24. Pfützner A, Schöndorf T, Hanefeld M, Forst T. High-sensitivity C-reactive protein predicts cardiovascular risk in diabetic and nondiabetic patients: effects of insulin-sensitizing treatment with pioglitazone. *J Diabetes Sci Technol* 2010; 4: 706-16.
25. Bassuk SS, Rifai N, Ridker PM High-sensitivity C-reactive protein: clinical importance. *Curr Probl Cardiol* 2004; 29: 439-93.
26. Rasouli N, Kern PA. Adipocytokines and the metabolic complications of obesity. *J Clin Endocrinol Metab* 2008; 93: S64-S73
27. Havel PJ. Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. *Curr Opin Lipidol* 2002; 13: 51-9.
28. Steppan CM, Bailey ST, Bhat S, et al. The hormone resistin links obesity to diabetes. *Nature* 2001; 409: 307-12.
29. Lewandowski KC, Szosland K, O'Callaghan C, Tan BK, Randeva HS, Lewinski A. Adiponectin and resistin serum levels in women with polycystic ovary syndrome during oral glucose tolerance test: a significant reciprocal correlation between adiponectin and resistin independent of insulin resistance indices. *Mol Genet Metab* 2005; 85: 61-9.
30. Wasim H, Al-Daghri NM, Chetty R, McTernan PG, Barnett AH, Kumar S. Relationship of serum adiponectin and resistin to glucose intolerance and fat topography in South-Asians. *Cardiovascular Diabetology* 2006; 5: 1-5.

Accepted: 15th April 2013
Correspondence:
Prof. Francesco Marotta, PhD
Piazza Firenze, 12
2014 Milano
E-mail: fmarchimede@libero.it