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Review

Endogenous Bioactive Peptides as Potential Biomarkers for Atherosclerotic Coronary Heart Disease

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Abstract: Cardiovascular disease is the leading cause of death worldwide, with high medical costs and rates of disability. It is therefore important to evaluate the use of cardiovascular biomarkers in the early diagnosis of coronary artery disease (CAD). We have screened a variety of recently identified bioactive peptides candidates in anticipation that they would allow detection of atherosclerotic CAD. Especially, we have focused on novel anti-atherogenic peptides as indicators and negative risk factors for CAD. *In vitro*, *in vivo* and clinical studies indicated that human adiponectin, heregulin-β_1, glucagon-like peptide-1 (GLP-1), and salusin-α, peptides of 244, 71, 30, and 28 amino acids, respectively, attenuate the development and progression of atherosclerotic lesions by suppressing macrophage foam cell formation via down-regulation of acyl-coenzyme A: cholesterol acyltransferase-1. Circulating levels of these peptides in the blood are significantly decreased in patients with CAD compared to patients without CAD. Receiver operating characteristic analyses showed that salusin-α is a more useful biomarker, with better sensitivity and specificity, compared with the others for detecting CAD. Therefore,

salusin-α, heregulin-β_1, adiponectin, and/or GLP-1, alone or in various combinations, may be useful as biomarkers for atherosclerotic CAD.

Keywords: acyl-coenzyme A: cholesterol acyltransferase-1; adiponectin; atherosclerosis; biomarker; coronary artery disease; glucagon-like peptide-1; heregulin-β_1; macrophage, salusin-α

1. Introduction

Over the past two decades, biomarkers have become increasingly utilized to improve overall patient care [1]. For example, biomarkers have had a significant impact in early detection of sub-clinical disease, diagnosis of acute or chronic syndromes, risk stratification, and in monitoring of disease and therapeutic efficacy [1]. Biomarkers are generally considered to be proteins or enzymes—measured in serum, plasma, or blood—that provide independent diagnostic and/or prognostic value by reflecting an underlying disease state [2].

Potential biomarkers have been extensively evaluated in the field of cardiovascular medicine as well as oncology [1]. Classical risk factors, such as lipids and glucose, have been well-established in coronary artery disease (CAD), while four additional markers have sufficient evidence of clinical utility to be recommended for regular clinical use: (1) cardiac troponin I and T; (2) B-type natriuretic peptides; (3) D-dimer; and (4) C-reactive protein (CRP) [1]. For example, epidemiological data demonstrated an association between high-sensitivity CRP and risk of future cardiovascular morbidity and mortality among those at high risk or with documented CAD [3].

However, only a limited number of markers have demonstrated significant diagnostic and/or therapeutic impact. Deeper insights into the pathophysiology of atherosclerosis have led to the discovery of additional novel biomarkers [1]. New vasoactive agents, inflammatory cytokines, and oxidative products that have attracted attentions have been implicated as potential biomarkers [1,2,4,5]. Previous studies have shown that high levels of pro-atherogenic vasoactive agents, such as serotonin and urotensin II, can be used as biomarkers for CAD [6,7]. Moreover, reduced circulating levels of anti-atherogenic vasoactive agents could also be used as indicators and/or negative risk factors for CAD [8,9]. In subsequent trials, we have focused on novel anti-atherogenic peptides; adiponectin, an adipocytokine [10], heregulin-β_1 (neuregulin-1 type I), a neuron growth factor [9], glucagon-like peptide-1 (GLP-1), an incretin hormone [11], and salusin-α, a peptide recently identified by an *in silico* approach [8].

Atherosclerosis is a pathological injury-to-response process that is initiated by early inflammatory responses of vascular endothelial cells [12]. Endothelial inflammation is characterized by decreased nitric oxide production, and monocyte adhesion and infiltration into the neointima lesion, followed by oxidized low-density lipoprotein (LDL)-induced transformation of macrophages into foam cells [12]. Vascular smooth muscle cell (VSMC) and fibroblast proliferation also plays an important role in the development of atherosclerotic lesions [12]. Therefore, any potent bioactive factors modulating such pathogenetic process could possibly be clinical atherosclerotic biomarkers.

This review focuses on the protective roles of adiponectin, heregulin-β_1, GLP-1, and salusin-α in atherosclerotic cardiovascular diseases and their emerging roles for biomarkers and therapeutic targets for CAD.

2. Roles in the Cardiovascular System

Human adiponectin, heregulin-β_1, GLP-1, and salusin-α are peptides of 244, 71, 30, and 28 amino acids, respectively. Adiponectin and GLP-1 are produced predominantly by adipose tissue and the L-cells of the lower gut, respectively, and less by the cardiovascular disease [10,13]. Salusin-α and heregulin-β_1 are both expressed in monocytes/macrophages, vascular endothelial cells, and VSMCs [9,14]. Receptors of adiponectin (AdipoR1 and AdipoR2), heregulin-β_1 (ErbB3 and ErbB4), and GLP-1 (GLP-1R) are abundantly expressed in human monocytes and macrophages [11,15,16], endothelial cells [10,17,18], VSMCs [11,19,20], and cardiomyocytes [21–23], while salusin-α receptors have not yet been identified [8,14].

As indicated in Table 1, adiponectin, heregulin-β_1, and GLP-1 suppress VSMC proliferation [11,20,24], show anti-inflammatory and anti-oxidant effects [18,25–29], and promote endothelial nitric oxide production [30–32]. Adiponectin, heregulin-β_1, and GLP-1 have been shown to exhibit cardioprotective effects against ischemic injury [33–35]. GLP-1 stimulates insulin secretin from pancreatic islet β-cells and lowers blood pressure [13]. GLP-1 and adiponectin are also known to ameliorate insulin resistance, lipid metabolism, and obesity [13,36]. Salusin-α has been shown to lower blood pressure, to promote mildly VSMC and fibroblast proliferation, and to suppress cardiomyocyte apoptosis, but no effect on endothelial nitric oxide production [14,37]. Other vasoactive effects of salusin-α have not yet been clarified [8].

Table 1. Effects of new novel peptides on the cardiovascular system.

	Adiponectin	**Heregulin-β_1**	**GLP-1**	**Salusin-α**
VSMC proliferation	↓	↓	↓	↑
eNOS	↑	↑	↑	→
Cardiomyocyte protection	+	+	+	+
Anti-inflammation	+	+	+	NE
Anti-oxidation	+	+	+	NE

GLP-1: glucagon-like peptide-1, VSMC: vascular smooth muscle cell, eNOS: endothelial nitric oxide synthase, NE: not examined. Arrows indicate either stimulation or suppression of VSMC proliferation and eNOS induction. + indicates positive effects.

3. Anti-Atherosclerotic Effects

Interestingly, adiponectin, heregulin-β_1, GLP-1, and salusin-α show common suppressive effects on macrophage-driven atherosclerosis. As listed in Table 2, adiponectin, heregulin-β_1, and salusin-α suppress foam cell formation, as indicated by cholesterol ester accumulation induced by acetylated LDL in primary cultured human monocyte-derived macrophages [16,38,39]. The intracellular free cholesterol level is increased by the endocytic uptake of acetylated LDL via scavenger receptor class A (SR-A) and is decreased by efflux of free cholesterol mediated by ATP-binding cassette transporter A1 (ABCA1) [12]. As excessive accumulation of free cholesterol is toxic to cells, free cholesterol must be

either removed through efflux to extracellular acceptors, such as apolipoprotein (apo) A1 and high-density lipoprotein, or esterified to cholesterol ester by the microsomal enzyme acyl-coenzyme A: cholesterol acyltransferase-1 (ACAT1) [12]. As indicated in Table 2, adiponectin, heregulin-β_1, and salusin-αsuppress ACAT1 expression in human monocyte-derived macrophages [16,38,39]. GLP-1 has been shown to suppress foam cell formation and ACAT1 expression in mouse macrophages [11]. Adiponectin and heregulin-β_1, but not salusin-α, suppress SR-A expression and enhance ABCA1 expression in human monocyte-derived macrophages [16,39–41] (Table 2). Adiponectin up-regulates ABCA1 via peroxisome proliferator-activated receptor-γ (PPARγ) and liver X receptor (LXR) signaling pathways in human macrophages [42].

Table 2. Effects of new novel peptides on macrophage foam cell formation.

	Adiponectin	**Heregulin-β_1**	**GLP-1**	**Salusin-α**
Foam cell formation	↓	↓	↓	↓
ACAT1	↓	↓	↓	↓
SR-A	↓	↓	→	→
ABCA1	↑	↑	→	→

GLP-1: glucagon-like peptide-1, ACAT1: acyl-coenzyme A: cholesterol acyltransferase-1, SR-A: scavenger receptor class A, ABCA1: ATP-binding cassette transporter A1. Arrows indicate stimulatory, suppressive, or negative effects.

Further, we and other groups have documented the anti-atherosclerotic effects of adiponectin, heregulin-β_1, GLP-1, and salusin-α by treatments of each peptide into apoE-knockout mice as an established animal model of atherosclerosis [11,16,43,44]. Treatments with adiponectin, heregulin-β_1, GLP-1, or salusin-α significantly attenuated aortic atherosclerotic lesions accompanied with a significant decrease in macrophage infiltration [11,16,43,44]. Significant suppressions of oxidized LDL-induced foam cell formation and ACAT1 expression were documented *ex vivo* in exudate peritoneal macrophages from apoE-knockout mice infused with GLP-1 or salusin-α compared with those from vehicle-infused apoE-knockout mice [11,44]. In these experiments, GLP-1 also downregulated CD36 that contributes to the endocytic uptake of oxidized LDL into macrophages [11]. Macrophage foam cells were less observed in aortic atherosclerotic lesions from adiponectin-transgenic LDL receptor-knockout mice fed with high-fat diet [45].

4. Presence in Coronary Artery Atherosclerosis and Circulating Blood

Immunohistochemical analyses of human coronary arteries from patients with CAD using anti-heregulin-β_1 or anti-salusin-α antibodies show faint staining in advanced coronary atherosclerotic lesions, suggesting decreased expression at their protein levels [16,39]. The expression of adiponectin at mRNA levels is also significantly lower in epicardial adipose tissues in CAD [46]. These findings strongly suggest that a decline in anti-atherogenic peptides may be associated with the progression of atherosclerotic lesions in human coronary arteries.

Circulating markers are more convenient for diagnosis of CAD. As specific antibodies against these peptides have been developed, their concentrations in blood samples could be quantified using radioimmunoassay and enzyme-linked immunosorbent assay (ELISA). Serum levels of total and

high-molecular weight adiponectin and plasma heregulin-β_1 levels were measured by ELISA, and their accuracy and precision were comparable among several studies [16,47–53]. The accuracy and precision of serum salusin-α and plasma GLP-1 levels measured by radioimmunoassay and ELISA were identical among each several studies [39,54–59].

To assess essential levels of peptide hormones, the factors that influence peptide production must be taken into consideration. In general, these include food intake, smoking, gender, and the presence of diabetes, hypertension or obesity. Serum salusin-α and plasma heregulin-β_1 levels have been demonstrated to be unaffected by a number of physiological stimuli [8,9]. Since adiponectin is known to show sexual dimorphism with higher levels in women than men, von Eynatten *et al*. [48] have studied serum adiponectin levels in the limited male subjects. Because GLP-1 is temporary increased after food intake, plasma GLP-1 levels have been measured in the fasting state and/or after 75-g oral glucose tolerance test [60–62]. Similar to adiponectin measurements, we determined serum salusin-α and plasma heregulin-β_1 levels in the fasting state [16,39].

5. Biomarkers for CAD

Matsubara *et al*. [60] reported that fasting plasma GLP-1 levels are significantly lower in CAD patients than in non-CAD patients (3.1 [2.4–3.6] *versus* 4.0 [3.1–5.9] pM, $P < 0.001$). Among patients without diabetes, the fasting plasma GLP-1 levels in CAD patients are significantly lower than in non-CAD patients (3.2 [2.6–3.7] *versus* 3.9 [3.0–5.2] pM, $P < 0.001$) [60]. However, Nathanson *et al*. [62] reported that impaired GLP-1 secretion after oral glucose load does not predict CAD in the presence of diabetes. El-Menyar *et al*. [63] reported that serum levels of high-molecular weight adiponectin are significantly decreased in CAD patients compared with those in non-CAD patients (1.9 ± 0.2 *versus* 3.1 ± 0.3 μg/mL, $P = 0.003$). Serum levels of high-molecular weight adiponectin were shown to be inversely correlated with angiographic severity of coronary artery lesions in patients with CAD [48]. Circulating levels of heregulin-β or salusin-α are also significantly decreased (Figure 1) and inversely correlated with angiographic severity of coronary artery lesions in patients with CAD [16,39,53].

Receiver operating characteristic (ROC) curves were plotted and the area under the curve (AUC) was analyzed to compare the predictive power of high-molecular weight adiponectin, heregulin-β_1, and salusin-α. The optimal cut-off values of these peptides for detecting CAD were set at the point showing a higher true-positive rate (sensitivity) with a low false-positive rate (1-specifity) on the respective ROC curve. von Eynatten *et al*. [48] reported that the AUC value for high-molecular weight adiponectin was 0.673. The ROC curve was slightly improved by using the high-molecular weight adiponectin/total adiponectin ratio, and its AUC value became 0.718. On the basis of our previous studies [16,39], we also performed ROC analyses and calculated the AUC values in the present time. The AUC values of heregulin-β_1 and salusin-α are 0.706 and 0.916, respectively (Figure 2). In addition, the cut-off levels were 2.4 ng/mL for heregulin-β_1 with sensitivity and specificity of 76.6% and 58.1%, respectively, and that for salusin-α was 8.5 pM with sensitivity and specificity of 81.5% and 92.7%, respectively. In comparisons among these peptides, the AUC values for high-molecular weight adiponectin and the high-molecular weight adiponectin/total adiponectin ratio were similar to that of heregulin-β_1 but inferior to that of salusin-α Therefore, serum salusin-α level has higher diagnostic value in detecting CAD compared with the other three peptides.

Figure 1. Comparisons of circulating heregulin-β_1 and salusin-α levels between CAD and non-CAD subjects. Peripheral venous blood was sampled from patients with angiographically proven CAD, acute coronary syndrome and stable angina pectoris, and non-CAD subjects including healthy volunteers and patients with mild hypertension [16,39]. Heregulin-β_1 and salusin-α were measured by ELISA and radioimmunoassay, respectively.

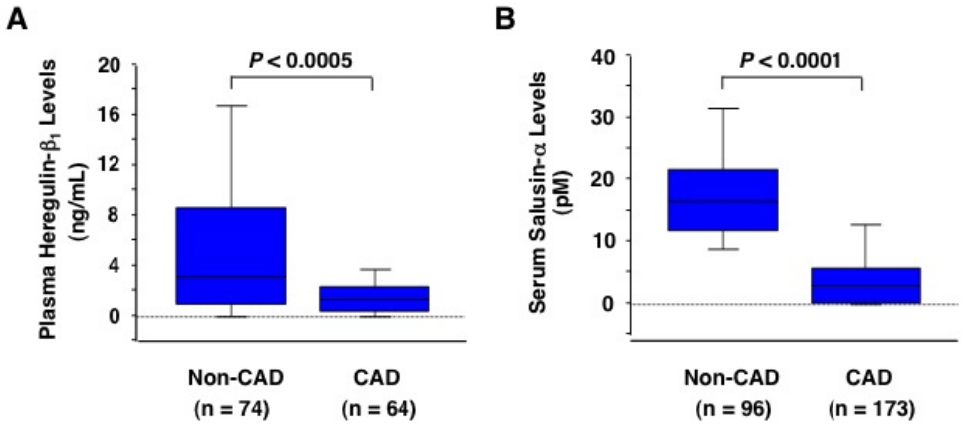
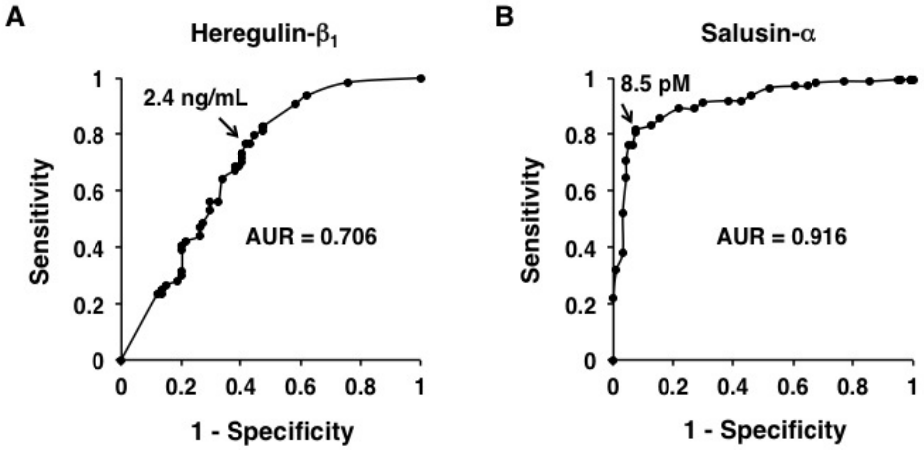


Figure 2. ROC curves of heregulin-β_1 and salusin-α for detecting CAD. Based on Figure 1 data from our previous studies [16,39], ROC analyses were performed and AUC was determined in respective ROC curve. The AUC value of salusin-α is greater than that of heregulin-β_1, indicating that salusin-α is more powerful marker for CAD than heregulin-β_1.



In patients with CAD, single biomarker shows somewhat high sensitivity and specificity, while the simultaneous measurement of a panel of biomarkers may increase the diagnostic accuracy [64]. Thus, these findings suggest that variously combined use of salusin-α with heregulin-β_1, adiponectin, GLP-1, and/or other biomarkers may become the still more powerful predictor for CAD.

6. Cardiac Dysfunction

Circulating levels of adiponectin, heregulon-β_1, GLP-1, and salusin-α were shown to be significantly associated with the severity of cardiac dysfunction [51,52,58,65]. Therefore, these peptides could also be potentially used as biomarkers reflecting heart failure. Elevated serum adiponectin and heregulon-β_1 levels are associated with adverse clinical outcomes in cases of cardiac

dysfunction [52,66]. Thus, further studies are required to determine their utility as biomarkers in predicting atherosclerotic CAD in the presence or absence of severe heart failure.

Recently, several studies have shown that administration of heregulin-β_1 or GLP-1 improves cardiac dysfunction in patients with heart failure [67–71]. Intracoronary administration of adiponectin led to a reduction in myocardial infarct size and improvement of left ventricular function after ischemia/reperfusion injury in pigs [72]. However, effects of adiponectin and salusin-α on cardiac function have not yet been reported in humans. These findings provide insights into the potential use of heregulin-β_1 or GLP-1 as an extended therapeutic window for combating refractory heart failure.

7. Conclusions

Adiponectin, heregulin-β_1, GLP-1, and salusin-α could contribute to the early diagnosis and therapeutic efficacy of atherosclerosis. Decreased levels of adiponectin, heregulin-β_1, GLP-1 and salusin-α in circulating blood and/or cardiovascular tissues are closely linked with human atherosclerosis. Thus, adiponectin, heregulin-β_1, GLP-1, and/or salusin-α, alone or in various combinations are candidate biomarkers for predicting CAD, which may be useful for the earlier detection of atherosclerotic cardiovascular diseases.

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References

1. Hochholzer, W.; Morrow, D.A.; Giugliano, R.P. Novel biomarkers in cardiovascular disease: Update 2010. *Am. J. Cardiol.* **2010**, *160*, 583–594.
2. Zakynthinos, E.; Pappa, N. Inflammatory biomarkers in coronary artery disease. *J. Cardiol.* **2009**, *53*, 317–333.
3. Corrado, E.; Rizzo, M.; Coppola, G.; Fattouch, K.; Novo, G.; Marturana, I.; Ferrara, F.; Novo, S. An update on the role of markers of inflammation in atherosclerosis. *J. Atheroscler. Thromb.* **2010**, *17*, 1–11.
4. Nagesh, C.M.; Roy, A. Role of biomarkers in risk stratification of acute coronary syndrome. *Indian J. Med. Res.* **2010**, *132*, 627–633.
5. Tsimikas, S. Oxidative biomarkers in the diagnosis and prognosis of cardiovascular disease. *Am. J. Cardiol.* **2006**, *98(Suppl.)*, 9–17.
6. Watanabe, T.; Koba, S. Roles of serotonin in atherothrombosis and related diseases. *Atherothrombosis* **2012**, in press.
7. Watanabe, T.; Arita, S.; Shiraishi, Y.; Suguro, T.; Sakai, T.; Hongo, S.; Miyazaki, A. Human urotensin II promotes hypertension and atherosclerotic cardiovascular diseases. *Curr. Med. Chem.* **2009**, *16*, 550–563.
8. Watanabe, T.; Sato, K.; Itoh, F.; Iso, Y.; Nagashima, M.; Hirano, T.; Shichiri, M. The roles of salusins in atherosclerosis and related cardiovascular diseases. *J. Am. Soc. Hypertens.* **2011**, *5*, 359–365.

9. Watanabe, T.; Sato, K.; Itoh, F.; Iso, Y. Pathogenic involvement of heregulin-β_1 in anti-atherogenesis. *Regul. Pept.* **2012**, *175*, 11–14.
10. Barseghian, A.; Gawande, D.; Bajaj, M. Adiponectin and vulnerable atherosclerotic plaques. *J. Am. Coll. Cardiol.* **2011**, *57*, 761–770.
11. Nagashima, M.; Watanabe, T.; Terasaki, M.; Tomoyasu, M.; Nohtomi, K.; Kim-Kaneyama, J.; Miyazaki, A.; Hirano, T. Native incretins prevent the development of atherosclerotic lesions in apolipoprotein E knockout mice. *Diabetologia* **2011**, *54*, 2649–2659.
12. Allahverdian, S.; Pannu, P.S.; Francis, G.A. Contribution of monocyte-derived macrophages and smooth muscle cells to arterial foam cell formation. *Cardiovasc. Res.* **2012**, in press.
13. Drucker, D.J. The biology of incretin hormones. *Cell Metab.* **2006**, *3*, 153–165.
14. Shichiri, M.; Ishimaru, S.; Ota, T.; Nishikawa, T.; Isogai, T.; Hirata, Y. Salusins: Newly identified bioactive peptides with hemodynamic and mitogenic activities. *Nat. Med.* **2003**, *9*, 1166–1172.
15. Chinetti, G.; Zawadski, C.; Fruchart, J.C.; Staels, B. Expression of adiponectin receptors in human macrophages and regulation by agonists of the nuclear receptors PPARα, PPARγ, and LXR. *Biochem. Biophys. Res. Commun.* **2004**, *314*, 151–158.
16. Xu, G.; Watanabe, T.; Iso, Y.; Koba, S.; Sakai, T.; Nagashima, M.; Arita, S.; Hongo, S.; Ota, H.; Kobayashi, Y.; *et al*. Preventive effects of heregulin-β_1 on macrophage foam cell formation and atherosclerosis. *Circ. Res.* **2009**, *105*, 500–510.
17. Russell, K.S.; Stern, D.F.; Polverini, P.J.; Bender, J.R. Neuregulin activation of ErbB receptors in vascular endothelium leads to angiogenesis. *Am. J. Physiol.* **1999**, *277*, H2205–H2211.
18. Arakawa, M.; Mita, T.; Azuma, K.; Ebato, C.; Goto, H.; Nomiyama, T.; Fujitani, Y.; Hirose, T.; Kawamori, R.; Watada, H. Inhibition of monocyte adhesion to endothelial cells and attenuation of atherosclerotic lesion by a glucagon-like peptide-1 receptor agonist, exendin-4. *Diabetes* **2010**, *59*, 1030–1037.
19. Ding, M.; Xie, Y.; Wagner, R.J.; Jin, Y.; Carrao, A.C.; Liu, L.S.; Guzman, A.K.; Powell, R.J.; Hwa, J.; Rzucidlo, E.M.; *et al*. Adiponectin induces vascular smooth muscle cell differentiation via repression of mammalian target of rapamycin complex 1 and FoxO4. *Arterioscler. Thromb. Vasc. Biol.* **2011**, *31*, 1403–1410.
20. Clement, C.M.; Thomas, L.K.; Mou, Y.; Croslan, D.R.; Gibbons, G.H.; Ford, B.D. Neuregulin-1 attenuates neointimal formation following vascular injury and inhibits the proliferation of vascular smooth muscle cells. *J. Vasc. Res.* **2007**, *44*, 303–312.
21. Ding, G.; Qin, Q.; He, N.; Francis-David, S.C.; Hou, J.; Liu, J.; Ricks, E.; Yang, Q. Adiponectin and its receptors are expressed in adult ventricular cardiomyocytes and upregulated by activation of peroxisome proliferator-activated receptor γ. *J. Mol. Cell. Cardiol.* **2007**, *43*, 73–84.
22. Iwamoto, R.; Yamazaki, S.; Asakura, M.; Takashima, S.; Hasuwa, H.; Miyado, K.; Adachi, S.; Kitakaze, M.; Hashimoto, K.; Raab, G.; *et al*. Heparin-binding EGF-like growth factor and ErbB signaling is essential for heart function. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 3221–3226.
23. Artinian, S.B.; Al Lafi, S.M.; Boutary, S.S.; Bitar, K.M.; Zwainy, N.S.; Bikhazi, A.B. Assessment of glucagon-like peptide-1 analogue and renin inhibitor on the binding and regulation of GLP-1 receptor in type 1 diabetic rat hearts. *Exp. Diabetes Res.* **2011**, *2011*, 489708.

24. Arita, Y.; Kihara, S.; Ouchi, N.; Maeda, K.; Kuriyama, H.; Okamoto, Y.; Kumada, M.; Hotta, K.; Nishida, M.; Takahashi, M.; *et al*. Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor induced common postreceptor signal in vascular smooth muscle cell. *Circulation* **2002**, *105*, 2893–2898.
25. Ouchi, N.; Walsh, K. Adiponectin as an anti-inflammatory factor. *Clinica. Chimica. Acta* **2007**, *380*, 24–30.
26. Xu, Z.; Ford, G.D.; Croslan, D.R.; Jiang, J.; Gates, A.; Allen, R.; Ford, B.D. Neuroprotection by neuregulin-1 following focal stroke is associated with the attenuation of ischemia-induced pro-inflammatory and stress gene expression. *Neurobiol. Dis.* **2005**, *19*, 461–470.
27. Motoshima, H.; Wu, X.; Mahadev, K.; Goldstein, B.J. Adiponectin suppresses proliferation and superoxide generation and enhances eNOS activity in endothelial cells treated with oxidized LDL. *Biochem. Biophys. Res. Commun.* **2004**, *315*, 264–271.
28. Timolati, F.; Ott, D.; Pentassuglia, L.; Giraud, M.N.; Perriard, J.C.; Suter, T.M.; Zuppinger, C. Neuregulin-1 beta attenuates doxorubicin-induced alterations of excitation-contraction coupling and reduces oxidative stress in adult rat cardiomyocytes. *J. Mol. Cell. Cardiol.* **2006**, *41*, 845–854.
29. Liu, F.Q.; Zhang, X.L.; Gong, L.; Wang, X.P.; Wang, J.; Hou, X.G.; Sun, Y.; Qin, W.D.; Wei, S.J.; Zhang, Y.; *et al*. Glucagon-like peptide 1 protects microvascular endothelial cells by inactivating the PARP-1/iNOS/NO pathway. *Mol. Cell. Endocrinol.* **2011**, *339*, 25–33.
30. Cheng, K.K.; Lam, K.S.; Wang, Y.; Huang, Y.; Carling, D.; Wu, D.; Wong, C.; Xu, A. Adiponectin-induced endothelial nitric oxide synthase activation and nitric oxide production are mediated by APPL1 in endothelial cells. *Diabetes* **2007**, *56*, 1387–1394.
31. Brero, A.; Ramella, R.; Fitou, A.; Dati, C.; Alloatti, G.; Gallo, M.P.; Levi, R. Neuregulin-1β1 rapidly modulates nitric oxide synthesis and calcium handling in rat cardiomyocytes. *Cardiovasc. Res.* **2010**, *88*, 443–452.
32. Ding, L.; Zhang, J. Glucagon-like peptide-1 activates endothelial nitric oxide synthase in human umbilical vein endothelial cells. *Acta Pharmacol. Sin.* **2012**, *33*, 75–81.
33. Shibata, R.; Izumiya, Y.; Sato, K.; Papanicolaou, K.; Kihara, S.; Colucci, W.S.; Sam, F.; Ouchi, N.; Walsh, K. Adiponectin protects against the development of systolic dysfunction following myocardial infarction. *J. Mol. Cell. Cardiol.* **2007**, *42*, 1065–1074.
34. Hedhli, N.; Huang, Q.; Kalinowski, A.; Palmeri, M.; Hu, X.; Russell, R.R.; Russell, K.S. Endothelium-derived neuregulin protects the heart against ischemic injury. *Circulation* **2011**, *123*, 2254–2262.
35. Nikolaidis, L.A.; Doverspike, A.; Hentosz, T.; Zourelias, L.; Shen, Y.T.; Elahi, D.; Shannon, R.P. Glucagon-like peptide-1 limits myocardial stunning following brief coronary occlusion and reperfusion in conscious canines. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 303–308.
36. Matsuzawa, Y. Adiponectin: A key player in obesity related disorders. *Curr. Pharm. Des.* **2010**, *16*, 1896–1901.
37. Yan, X.H.; Li, L.; Pan, Y.X.; Lu, H.; Rong, W.F.; Yan, L.; Ren, A.J.; Tang, C.S.; Yuan, W.J. Salusins protect neonatal rat cardiomyocytes from serum deprivation-induced cell death through upregulation of GRP78. *J. Cardiovasc. Pharmacol.* **2006**, *48*, 41–46.

38. Furukawa, K.; Hori, M.; Ouchi, N.; Kihara, S.; Funahashi, T.; Matsuzawa, Y.; Miyazaki, A.; Nakayama, H.; Horiuchi, S. Adiponectin down-regulates acyl-coenzyme A: Cholesterol acyltransferase-1 in cultured human monocyte-derived macrophages. *Biochem. Biophys. Res. Commun.* **2004**, *317*, 831–836.
39. Watanabe, T.; Nishio, K.; Kanome, T.; Matsuyama, T.; Koba, S.; Sakai, T.; Sato, K.; Hongo, S.; Nose, K.; Ota, H.; *et al*. Impact of salusin-α and -β on human macrophage foam cell formation and coronary atherosclerosis. *Circulation* **2008**, *117*, 638–648.
40. Ouchi, N.; Kihara, S.; Arita, Y.; Nishida, M.; Matsuyama, A.; Okamoto, Y.; Ishigami, M.; Kuriyama, H.; Kishida, K.; Nishizawa, H.; *et al*. Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. *Circulation* **2001**, *103*, 1057–1063.
41. Tsubakio-Yamamoto, K.; Matsuura, F.; Koseki, M.; Oku, H.; Sandoval, J.C.; Inagaki, M.; Nakatani, K.; Nakaoka, H.; Kawase, R.; Yuasa-Kawase, M.; *et al*. Adiponectin prevents atherosclerosis by increasing cholesterol efflux from macrophages. *Biochem. Biophys. Res. Commun.* **2008**, *375*, 390–394.
42. Tian, L.; Luo, N.; Klein, R.L.; Chung, B.H.; Garvey, W.T.; Fu, Y. Adiponectin reduces lipid accumulation in macrophage foam cells. *Atherosclerosis* **2009**, *202*, 152–161.
43. Okamoto, Y.; Kihara, S.; Ouchi, N.; Nishida, M.; Arita, Y.; Kumada, M.; Ohashi, K.; Sakai, N.; Shimomura, I.; Kobayashi, H.; *et al*. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation* **2002**, *106*, 2767–2770.
44. Nagashima, M.; Watanabe, T.; Shiraishi, Y.; Morita, R.; Terasaki, M.; Arita, S.; Hongo, S.; Sato, K.; Shichiri, M.; Miyazaki, A.; *et al*. Chronic infusion of salusin-α and -β exerts opposite effects on atherosclerotic lesion development in apolipoprotein E-deficient mice. *Atherosclerosis* **2010**, *212*, 70–77.
45. Luo, N.; Liu, J.; Chung, B.H.; Yang, Q.; Klein, R.L.; Garvey, W.T.; Fu, Y. Macrophage adiponectin expression improves insulin sensitivity and protects against inflammation and atherosclerosis. *Diabetes* **2010**, *59*, 791–799.
46. Zhou, Y.; Wei, Y.; Wang, L.; Wang, X.; Du, X.; Sun, Z.; Dong, N.; Chen, X. Decreased adiponectin and increased inflammation expression in epicardial adipose tissue in coronary artery disease. *Cardiovasc. Diabetol.* **2011**, *10*, 2.
47. Nakano, Y.; Tajima, S.; Yoshimi, A.; Akiyama, H.; Tsushima, M.; Tanioka, T.; Negoro, T.; Tomita, M.; Tobe, T. A novel enzyme-linked immunosorbent assay specific for high-molecular-weight adiponectin. *J. Lipid Res.* **2006**, *47*, 1572–1582.
48. von Eynatten, M.; Humpert, P.M.; Bluemm, A.; Lepper, P.M.; Hamann, A.; Allolio, B; Nawroth, P.P.; Bierhaus, A.; Dugi, K.A. High-molecular weight adiponectin is independently associated with the extent of coronary artery disease in men. *Atherosclerosis* **2008**, *99*, 123–128.
49. Liang, K.W.; Sheu, W.H.; Lee, W.L.; Liu, T.J.; Ting, C.T.; Hsieh, Y.C.; Wang, K.Y.; Chen, Y.T.; Lee, W.J. Decreased circulating protective adiponectin level is associated with angiographic coronary disease progression in patients with angina pectoris. *Int. J. Cardiol.* **2008**, *129*, 76–80.
50. Broedl, U.C.; Lebherz, C.; Lehrke, M.; Stark, R.; Greif, M.; Becker, A.; von Ziegler, F.; Tittus, J.; Reiser, M.; Becker, C.; *et al*. Low adiponectin levels are an independent predictor of mixed and non-calcified coronary atherosclerotic plaques. *PLoS One* **2009**, *4*, e4733.

51. Baldasseroni, S.; Mannucci, E.; Orso, F.; Di Serio, C.; Pratesi, A.; Bartoli, N.; Marella, G.A.; Colombi, C.; Foschini, A.; Valoti, P.; *et al*. Adiponectin in outpatients with coronary artery disease: Independent predictors and relationship with heart failure. *Nutr. Metab. Cardiovasc. Dis.* **2012**, *22*, 292–299.
52. Ky, B.; Kimmel, S.E.; Safa, R.N.; Putt, M.E.; Sweitzer, N.K.; Fang, J.C.; Sawyer, D.B.; Cappola, T.P. Neuregulin-1β is associated with disease severity and adverse outcomes in chronic heart failure. *Circulation* **2009**, *120*, 310–317.
53. Geisberg, C.A.; Wang, G.; Safa, R.N.; Smith, H.M.; Anderson, B.; Peng, X.Y.; Veerkamp, B.; Zhao, D.X.; Blakemore, D.; Yu, C.; *et al*. Circulating neuregulin-1β levels vary according to the angiographic severity of coronary artery disease and ischemia. *Coron. Artery Dis.* **2011**, *22*, 577–582.
54. Sato, K.; Koyama, T.; Tateno, T.; Hirata, Y.; Shichiri, M. Presence of immunoreactive salusin-α in human serum and urine. *Peptides* **2006**, *27*, 2561–2566.
55. Watanabe, T.; Suguro, T.; Sato, K.; Koyama, T.; Nagashima, M.; Kodate, S.; Hirano, T.; Adachi, M.; Shichiri, M.; Miyazaki, A. Serum salusin-α levels are decreased and correlated negatively with carotid atherosclerosis in essential hypertensive patients. *Hypertens. Res.* **2008**, *31*, 463–468.
56. Kimoto, S.; Sato, K.; Watanabe, T.; Suguro, T.; Koyama, T.; Shichiri, M. Serum levels and urinary excretion of salusin-α in renal insufficiency. *Regul. Pept.* **2010**, *162*, 129–132.
57. Ozgen, M.; Koca, S.S.; Dagli, N.; Balin, M.; Ustundag, B.; Isik, A. Serum salusin-alpha level in rheumatoid arthritis. *Regul. Pept.* **2010**, *167*, 125–128.
58. Ti, Y.; Wang, F.; Wang, Z.H.; Wang, X.L.; Zhang, W.; Zhang, Y.; Bu, P.L. Associations of serum salusin-α levels with atherosclerosis and left ventricular diastolic dysfunction in essential hypertension. *J. Hum. Hypertens.* **2012**, in press.
59. Simsek, Y.; Celik, O.; Yilmaz, E.; Karaer, A.; Dogan, C.; Aydin, S.; Ozer, A. Serum levels of apelin, salusin-alpha, and salusin-beta in normal pregnancy and preeclampsia. *J. Matern. Fetal. Neonatal. Med.* **2012**, in press.
60. Matsubara, J.; Sugiyama, S.; Sugamura, K.; Nakamura, T.; Fujiwara, Y.; Akiyama, E.; Kurokawa, H.; Nozaki, T.; Ohba, K.; Konishi, M.; *et al*. A dipeptidyl peptidase-4 inhibitor, des-fluoro-sitagliptin, improves endothelial function and reduces atherosclerotic lesion formation in apolipoprotein E-deficient mice. *J. Am. Coll. Cardiol.* **2012**, *59*, 265–276
61. Lee, S.; Yabe, D.; Nohtomi, K.; Morita, R.; Seino, Y.; Hirano, T. Intact glucagon-like peptide-1 levels are not decreased in Japanese patients with type 2 diabetes. *Endocr. J.* **2011**, *57*, 119–126.
62. Nathanson, D.; Zethelius, B.; Berne, C.; Holst, J.J.; Sjöholm, A.; Nyström, T. Reduced plasma levels of glucagon-like peptide-1 in elderly men are associated with impaired glucose tolerance but not with coronary heart disease. *Diabetologia* **2010**, *53*, 277–280.
63. El-Menyar, A.; Rizk, N.; Al Nabti, A.D.; Hassira, S.A.; Singh, R.; Abdel Rahman, M.O.; Suwaidi, J.A. Total and high molecular weight adiponectin in patients with coronary artery disease. *J. Cardiovasc. Med.* **2009**, *10*, 310–315.
64. Brodov, Y.; Behar, S.; Goldenberg, I.; Boyko, V.; Chouraqui, P. Usefulness of combining serum uric acid and C-reactive protein for risk stratification of patients with coronary artery disease (Bezafibrate Infarction Prevention [BIP] study). *Am. J. Cardiol.* **2009**, *104*, 194–198.
65. Nathanson, D.; Zethelius, B.; Berne, C.; Lind, L.; Andrén, B.; Ingelsson, E.; Holst, J.J.; Nyström, T. Plasma levels of glucagon like peptide-1 associate with diastolic function in elderly men. *Diabet. Med.* **2011**, *28*, 301–305.

66. Kojima, S.; Funahashi, T.; Otsuka, F.; Maruyoshi, H.; Yamashita, T.; Kajiwara, I.; Shimomura, H.; Miyao, Y.; Fujimoto, K.; Sugiyama, S.; *et al*. Future adverse cardiac events can be predicted by persistently low plasma adiponectin concentrations in men and marked reductions of adiponectin in women after acute myocardial infarction. *Atherosclerosis* **2007**, *194*, 204–213.
67. Gao, R.; Zhang, J.; Cheng, L.; Wu, X.; Dong, W.; Yang, X.; Li, T.; Liu, X.; Xu, Y.; Li, X.; *et al*. A phase II, randomized, double-blind, multicenter, based on standard therapy, placebo-controlled study of the efficacy and safety of recombinant human neuregulin-1 in patients with chronic heart failure. *J. Am. Coll. Cardiol.* **2010**, *55*, 1907–1914.
68. Jabbour, A.; Hayward, C.S.; Keogh, A.M.; Kotlyar, E.; McCrohon, J.A.; England, J.F.; Amor, R.; Liu, X.; Li, X.Y.; Zhou, M.D.; *et al*. Parenteral administration of recombinant human neuregulin-1 to patients with stable chronic heart failure produces favourable acute and chronic haemodynamic responses. *Eur. J. Heart Fail.* **2011**, *13*, 83–92.
69. Sokos, G.G.; Nikolaidis, L.A.; Mankad, S.; Elahi, D.; Shannon, R.P. Glucagon-like peptide-1 infusion improves left ventricular ejection fraction and functional status in patients with chronic heart failure. *J. Card. Fail.* **2006**, *12*, 694–699.
70. Read, P.A.; Hoole; S.P.; White, P.A.; Khan, F.Z.; O'Sullivan, M.; West, N.E.; Dutka, D.P. A pilot study to assess whether glucagon-like peptide-1 protects the heart from ischemic dysfunction and attenuates stunning after coronary balloon occlusion in humans. *Circ. Cardiovasc. Interv.* **2011**, *4*, 266–272.
71. Read, P.A.; Khan, F.Z.; Dutka, D.P. Cardioprotection against ischemia induced by dobutamine stress using glucagon-like peptide-1 in patients with coronary artery disease. *Heart* **2012**, *98*, 408–413.
72. Kondo, K.; Shibata, R.; Unno, K.; Shimano, M.; Ishii, M.; Kito, T.; Shintani, S.; Walsh, K.; Ouchi, N.; Murohara, T. Impact of a single intracoronary administration of adiponectin on myocardial ischemia/reperfusion injury in a pig model. *Circ. Cardiovasc. Interv.* **2010**, *3*, 166–173.