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Intrinsic disorder in biomarkers of insulin resistance, hypoadiponectinemia, and endothelial dysfunction among the type 2 diabetic patients

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a chronic and progressive disease that is strongly associated with various complications including cardiovascular diseases and related mortality. The present study aimed to analyze the abundance and functionality of intrinsically disordered regions in several biomarkers of insulin resistance, adiponectin, and endothelial dysfunction found in the T2DM patients. In fact, in comparison to controls, obese T2DM patients are known to have significantly higher levels of inter-cellular adhesion molecule (iCAM-1), vascular cell adhesion molecule (vCAM-1), and E-selectin, whereas their adiponectin levels are relatively low. Bioinformatics analysis revealed that these selected biomarkers (iCAM-1, vCAM-1, E-selectin, and adiponectin) are characterized by the noticeable levels of intrinsic disorder propensity and high binding promiscuity, which are important features expected for proteins serving as biomarkers. Within the limit of studied groups, there is an association between insulin resistance and both hypoadiponectinemia and endothelial dysfunction.

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Introduction

The world-wide ubiquity of diabetes is disturbingly high and is growing. Here are just a few illustrative facts: if in the year of 2000 there were 171 million people with diabetes worldwide, this number is expected to upsurge to 366 million by 2030.¹ In another study, the national levels of diabetes prevalence among adults (aged 20–79 years) of 216 countries in 2010 were evaluated and used to make a projection for 2030.² This analysis revealed that in 2010, the world prevalence of diabetes was 6.4% and this affliction affected 285 million adult patients, whereas by 2030, the diabetes pervasiveness is expected to rise to 7.7% affecting 439 million adults.² It is also expected that by 2030, there will be 69% and 20% increase in the numbers of adults with diabetes in developing and developed countries, respectively.²

Among other health threats, type 2 diabetes mellitus (T2DM) is an important cardiovascular disease (CVD) risk factor that causes the reduction of the life

expectancy^{3,4} and is accompanied by hypertension.⁵ T2DM is often associated with obesity brought about by the increased adipose tissue mass originating from the increase in the number and size of adipocytes.⁶ In addition, T2DM is typically associated with the reduced levels of high density lipoprotein (HDL) cholesterol (HDL-C) and is characterized by the HDL functional impairment.^{7,8}

Being an active endocrine organ, adipose tissue is known to secrete various adipocytokines or adipokines, which are biologically active substances involved in the local and systemic regulation of numerous metabolic and inflammatory processes.⁹ When this endocrine function of the adipose tissue is deregulated, serious obesity-related metabolic disorders start to develop including T2DM, insulin resistance, and atherosclerosis.¹⁰

Among the variety of adipokines secreted by the adipose tissue is an important protein adiponectin, which is known to have the insulin sensitizing and

anti-inflammatory activities, is able to improve systemic glucose tolerance, and has vasodilatory function protecting the vasculature from atherosclerosis.¹¹ In the insulin-resistant humans and animals,¹² as well as in obesity and in the patients with T2DM,¹³ the circulating levels of adiponectin are decreased. Therefore, hypo adiponectinemia is considered as an independent risk factor for T2DM and CVD development.¹⁴

Overall, there is a close association between the T2DM incidence, insulin resistance, the abnormal levels of inflammatory markers,¹³ and the adiponectin circulating levels. In fact, it is believed that the abnormal levels of inflammatory markers and the endothelial cell dysfunction^{15,16} associated with hyperlipidemia, hyperinsulinemia, and pancreatic β -cell failure¹⁶ are typical for the T2DM patients. Furthermore, endothelial dysfunction is characterized by the prothrombotic properties, pro-inflammatory state, and reduced vasodilation.^{17,18} One of the reasons for the association between the endothelial dysfunction and insulin resistance is an essential role played by insulin in the vascular function regulation via stimulation of the expression of vascular cell adhesion molecules, such as soluble vascular cell adhesion molecule-1 (vCAM-1), soluble intercellular cell adhesion molecule-1 (iCAM-1), and E-selectin on endothelium.¹⁹

Further support of the importance of adiponectin in regulation of the endothelial function is given by Lisowska *et al.*, who found that in the angina pectoris patients undergoing coronary artery bypass grafting (CABG) the concentrations of adiponectin and cell adhesion molecule CD146 before the surgery were significantly lower than those in the control group.²⁰ However, three months after the CABG, the adiponectin and CD146 levels were significantly increased, correlating with the concentrations of thrombomodulin, which is a natural antithrombin glycoprotein, and the well-established endothelial dysfunction marker, Von Willebrand factor.²⁰ These findings suggested that adiponectin and CD146 can serve as markers of endothelial cell dysfunction,^{20,21} and that the cardiovascular system can be protected by the high adiponectin levels. However, no association between the adiponectin levels and the development of coronary artery disease was found in another study.²² To address some of these controversies, our study aimed to detect an association between insulin resistance, adiponectin, and endothelial dysfunction biomarkers and their intrinsic disorder status.

Recent studies revealed that many proteins with biologically important functions do not possess unique 3D-structures.²³⁻³³ These intrinsically disordered proteins (IDPs) and hybrid proteins containing both ordered and intrinsically disordered domains/regions³⁴ are very common in nature,^{31,35-39} with IDPs constituting significant fractions of all known proteomes,^{31,35,36,40,41} and therefore being considered now as an important extension of the protein kingdom.^{23,28,32,42-46} Despite their lack of unique 3D-structures, these proteins are involved in crucial biological processes (such as signaling, regulation, and recognition),^{23-29,31,42,47-58} and their functions complement the functional repertoire of the ordered proteins.⁵⁵⁻⁵⁷ Furthermore, being general regulators and controllers, many IDPs are intimately associated with the variety of human diseases.^{59,60} Since earlier study revealed that proteins related to CVD are enriched in intrinsic disorder,⁶¹ and since T2DM is an important CVD risk factor, we analyzed the intrinsic disorder propensity and the presence of disorder-based functional sites in the biomarkers utilized in this study (human adiponectin, iCAM-1, vCAM-1, and E-selectin) by a series of bioinformatics tools.

Materials and methods

Computational analyses of the amino acid sequences of biomarkers

Amino acid sequences of four human proteins utilized as biomarkers, adiponectin (UniProt ID: Q15848), inter-cellular adhesion molecule (iCAM-1, UniProt ID: P05362), vascular cell adhesion molecule (vCAM-1, UniProt ID: P19320), and E-selectin (UniProt ID: P16581) were downloaded from UniProt (<http://www.uniprot.org/uniprot/>). The intrinsic disorder propensities of these biomarkers were evaluated by several per-residues disorder predictors, such as PONDR® VLXT,⁶² PONDR® VSL2,⁶³ PONDR® VL3,⁶⁴ and PONDR® FIT,⁶⁵ which were chosen based on their different sensitivities to the various protein intrinsic disorder-related features. PONDR® VSL2B is one of the more accurate stand-alone disorder predictors,^{63,66,67} PONDR® VLXT is known to have high sensitivity to local sequence peculiarities and can be used for identifying disorder-based interaction sites,⁶² whereas a metapredictor PONDR-FIT⁶⁵ is moderately more accurate than each of its component predictors,

PONDR[®] VLXT,⁶² PONDR[®] VSL2,⁶³ PONDR[®] VL3,⁶⁴ FoldIndex,⁶⁸ and IUPred.⁶⁹

The D²P² internet database,⁷⁰ which is a community resource for the pre-computed disorder predictions on a large library of proteins from completely sequenced genomes (<http://d2p2.pro/>) was used to provide more information on the presence of functional disordered regions in the query proteins. As the measure of disorder predisposition, D²P² database uses outputs of PONDR[®] VLXT,⁶² IUPred,⁶⁹ PONDR[®] VSL2B,^{71,72} PrDOS,⁷³ ESpritz,⁷⁴ and PV2.⁷⁰ This database is further enhanced by the information on the curated sites of various posttranslational modifications and on the location of predicted disorder-based potential binding sites.

Finally, the interactivity of biomarkers used in this study was further evaluated by STRING (Search Tool for the Retrieval of Interacting Genes) database, which is the online resource that provides both experimental and predicted interaction information for query proteins.⁷⁵

Results

As it follows from literature data and our own results (data not shown) in comparison with controls, T2DM patients typically show significantly higher levels of iCAM-1, vCAM-1, and E-selectin, whereas their adiponectin levels are significantly lower than those of the matched controls. To check the intrinsic disorder status of the biomarkers used in our study, we evaluated the disorder propensities of human adiponectin, iCAM-1, vCAM-1, and E-selectin by several per-residues disorder predictors. Results of this analysis are summarized in Figure 1, which clearly shows that the N-terminal half of adiponectin is predicted to be highly disordered, and that all other biomarkers contain substantial amounts of disorder, mostly in the form of short disordered regions.

Next, the disorder predispositions of these proteins were analyzed by the D²P² database.⁷⁰ Figure 2 represents the results of this analysis and confirms that

Figure 1. Evaluating the intrinsic disorder propensities of the human adiponectin (UniProt ID: Q15848; A), iCAM-1 (UniProt ID: P05362; B), vCAM-1 (UniProt ID: P19320; C), and E-selectin (UniProt ID: P16581; D) by the family of PONDR predictors. A disorder threshold is indicated as a thin line (at score = 0.5) in all plots to show a boundary between disorder (>0.5) and order (<0.5).

Figure 2. Evaluation of the functional intrinsic disorder propensity of the human adiponectin (UniProt ID: Q15848; A), iCAM-1 (UniProt ID: P05362; B), vCAM-1 (UniProt ID: P19320; C), and E-selectin (UniProt ID: P16581; D) by the D²P² platform (<http://d2p2.pro/>).⁷⁰ In this plot, top nine colored bars represent location of disordered regions predicted by different computational tools (Espritz-D, Espritz-N, Espritz-X, IUPred-L, IUPred-S, PV2, PrDOS, POND^R VSL2b, and POND^R VLXT, see keys for the corresponding color codes). Dark red bar shows the location of the functional domain found by the Pfam platform, which is a database of protein families that includes their annotations and multiple sequence alignments generated using hidden Markov models.¹³⁷⁻¹³⁹ Green-and-white bar in the middle of the plot shows the predicted disorder agreement between these nine predictors, with green parts corresponding to disordered regions by consensus. Red, yellow and purple circles at the bottom of the plot show the locations of phosphorylation, acetylation and ubiquitination sites, respectively.

human adiponectin, iCAM-1, vCAM-1, and E-selectin all possess functional disordered regions containing potential phosphorylation and protein-protein interaction sites. These observations are in agreement with the well-known fact that phosphorylation sites⁷⁶ and sites of various posttranslational modifications

(PTMs) are preferentially located within the intrinsically disordered regions.⁷⁷

Finally, Figure 3 represents the outputs of the STRING online computational platform⁷⁵ that illustrate the interactability of human adiponectin, iCAM-1, vCAM-1, and E-selectin and shows that these

Figure 3. Analysis of the interactivity of the human adiponectin (UniProt ID: Q15848; A), iCAM-1 (UniProt ID: P05362; B), vCAM-1 (UniProt ID: P19320; C), and E-selectin (UniProt ID: P16581; D) by STRING.⁷⁵ STRING produces the network of predicted associations for a particular group of proteins. The network nodes are proteins, whereas the edges represent the predicted or known functional associations. An edge may be drawn with up to 7 differently colored lines that represent the existence of the seven types of evidence used in predicting the associations. A red line indicates the presence of fusion evidence; a green line - neighborhood evidence; a blue line - co-occurrence evidence; a purple line - experimental evidence; a yellow line - text mining evidence; a light blue line - database evidence; a black line - co-expression evidence.⁷⁵

biomarkers are characterized by high binding promiscuity.

It is important to note here that the intrinsic disorder propensities described here for the T2DM biomarkers might have significant functional implementations. In fact, some previous studies do indicate the potential roles of intrinsically disordered regions in functionality of these proteins and in their involvement into pathogenesis. For example, based on the PONDR[®] VSL2 analysis, human adiponectin

(UniProt ID: Q15848) is predicted to have the average disorder score of 0.477 and have 6 disordered regions that include 45.9% of all its residues. Structurally, adiponectin can be divided into the N-terminal variable region (residues 19–41), the collagenous domain (residues 42–107), and the C-terminal globular domain (residues 108–244).^{78,79} This protein is known to exist in various oligomeric forms including a trimer, a hexamer and larger order multimeric forms collectively known as high molecular weight multimers

(HMW).⁸⁰ Formation of trimers is driven by the hydrophobic interactions among the globular domains and a propensity of the collagenous domains to form the triple helical structure,^{78,81,82} whereas multimers larger than a trimer are stabilized by a disulfide bond between two trimers via a conserved Cys₂₂ residue in the variable region,^{80,83} and the formation of HMW multimers larger than a hexamer requires specific post-translational modifications (PTMs).⁸⁴ Furthermore, G84R and G90S mutants of adiponectin that cannot form HMW multimers are known to be associated with diabetes and hypoadiponectinemia.⁸⁰ Noticeably, the multimerization-promoting PTMs (e.g., glycosylation sites at Lys₆₈, Lys₇₁, Lys₈₀, and Lys₁₀₄, and hydroxylation on Pro₇₁, Pro₇₆, and Pro₉₅) and multimerization-inhibiting mutations are all located within the N-terminal half of adiponectin that contains the N-terminal variable region and the collagenous domain and is predicted to be mostly disordered in our study (see Figs. 1 and 2). In our view, these observations provide a direct link between the intrinsically disordered nature of the N-terminal domain of adiponectin and the role of this protein in diabetes and hypoadiponectinemia. Finding of the significant amounts of disorder in collagenous domain of adiponectin is in agreement with earlier studies, where very high levels of disorder were found in various extracellular matrix proteins and in collegens.^{85,86}

Human E-selectin (UniProt ID: P16581) is predicted by PONDR[®] VSL2 to have 42.0% disordered residues, 8 disordered regions, and the average disorder score of 0.456. This protein possesses an N-terminal Ca²⁺-dependent lectin domain (residues 22–139), an epidermal growth factor (EGF)-like domain (residues 140–175), six consensus repeat units, Sushi domains, also known as Complement control protein (CCP) modules, or short consensus repeats (SCR) (residues 178–239, 240–301, 303–364, 366–427, 429–490, and 491–549), a transmembrane (TM) domain (residues 557–578), and an intracellular cytoplasmic C-tail (residues 579–610). Functional analysis showed that the short consensus repeat (SCR) domains are needed for the efficient interaction of this protein with its ligand that all six SCR domains play a role in blocking neutrophil or HL-60 cell adhesion to E-selectin.⁸⁷ Figures 1 and 2 shows that there are several disordered regions in E-selectin (e.g., according to the PONDR[®] VSL2 analysis, this protein has five relatively long disordered regions, residues 185–233, 281–

350, 372–423, 459–493, and 584–610) that cover significant portions of functional Sushi domains and the cytoplasmic C-tail.

According to the PONDR[®] VSL2 analysis, human iCAM-1 (UniProt ID: P19320) is predicted to have 36.1 % disordered residues assembled into 11 disordered regions and have an average disorder score of 0.443. Besides a transmembrane spanning region (residues 481–503) and a short cytoplasmic domain (residues 504–532), there are five extracellular Ig-like C2-type domains (D1–D5) in human iCAM-1 (residues 41–103, 128–193, 230–297, 325–378, and 412–464). Figures 1B and 2B show that although each of these IG-like domains is expected to have significant levels of intrinsic disorder, their intrinsic disorder propensities are not identical. In fact, domains D1 and D4 are predicted to have the most disordered residues followed by domain D2, and with domains D3 and D5 possessing least amount of disorder. The Ig-like domains are known to have different functions. For example, D1 serves as the binding site for the integrin lymphocyte function-associated antigen-1 (LFA-1) and human rhinovirus,⁸⁸ whereas D3 binds to the Mac-1 integrin.⁸⁹ Human iCAM-1 is predicted to have 6 disorder-based potential binding sites (molecular recognition features, MoRFs) and a highly disordered cytoplasmic C-tail, which is heavily decorated with multiple PTMs. Furthermore, domains D2, D3, and D4 of iCAM-1 were shown to contain a number of N-linked glycan chains that contribute to stability and function of this protein.^{90–92} Known glycosylation sites (GlcNAc residues) are located at positions 130 (0.285), 145 (0.470), 183 (0.441), 202 (0.454), 260 (0.333), 267 (0.460), 296 (0.306), 385 (0.359), and 406 (0.414) and are characterized by relatively large disorder scores (numbers shown in brackets), suggesting that all these sites are located within the flexible regions. Curiously, in agreement with our finding that D1 is expected to be one of the most disordered domains of iCAM-1, it was shown that this domain cannot fold on its own likely because of the loss of its interaction with the second domain.⁸⁸

Although Figures 1 and 2 show that human vCAM-1 (UniProt ID: P19320) is predicted to be the most ordered of the T2DM biomarkers analyzed in our study, this protein has several disordered and flexible regions characterized by the disorder scores exceeding 0.5 and 0.3, respectively. In fact, according to the PONDR[®] VSL2 analysis, this protein has an average

disorder score of 0.384 and contains 22.1% disordered residues assembled into 16 relatively short disordered regions. There are seven extracellular, Ig-like C2-type domains in this protein (residues 25–105, 109–212, 223–309, 312–399, 408–506, 511–595, and 600–684), C-terminally located transmembrane region (residues 699–720) and a very short cytoplasmic C-tail (residues 721–739). Importantly, almost all linkers between these domains are predicted to be disordered or flexible. Furthermore, intrinsic disorder propensities of individual Ig-like C2-type domains are not even, with three of these domains (residues 312–399, 408–506 and 511–595) being definitely more disordered than their counterparts (see Figs. 1C and 2C). It is known that vCAM-1 can be found in three isoforms produced by alternative splicing, where isoforms 2 and 3 differ from the canonical isoform shown in Figure 1C and 2C by missing the 310–402 (which is substituted by a single alanine residue) and 52–113 regions, respectively. In agreement with earlier study showing that the mRNA regions affected by alternative splicing are significantly biased toward encoding disordered residues,⁹³ these two regions of vCAM-1 removed by alternative splicing are predicted to contain significant levels of disorder.

Discussion

The hyperglycemia caused in T2DM by the insulin resistance can eventually cause the development of a multitude of the micro- and macrovascular complications. As a result, in comparison with healthy controls, the T2DM patients experience two to four times higher risk of the development of coronary artery disease,⁹⁴ peripheral vascular disease,⁹⁵ and cerebrovascular disease.⁹⁶ Furthermore, CVD-related mortality among the patients with T2DM may be up to four times higher than that seen in the background population.⁹⁷ It is known that insulin resistance and its manifestations precede T2DM and its cardiovascular complications and therefore can be used to predict these maladies.⁹⁸ Furthermore, there is an association between the insulin resistance characterizing the obese subjects and the endothelial dysfunction.^{99,100} Also, the obese individuals are characterized by the down regulation of an important circulating adipose tissue-derived hormone, adiponectin.¹⁰¹ Experimental studies show that adiponectin may protect against the

development of insulin resistance, atherosclerosis, and inflammation.¹⁰²

Therefore, it is likely that the obese T2DM patients have insulin resistance and are characterized by the reduction of the adiponectin levels and alterations in the levels of the endothelial function biomarkers. This hypothesis is in agreement with the work by Meigs et al., who reported that the endothelial dysfunction can be used to predict T2DM among women.¹⁰³ Also, Thorand et al. supported the role of the endothelial dysfunction in the T2DM pathogenesis.¹⁰⁴ The levels of soluble E-selectin were independently found to be associated with diabetes.^{105,106} In addition, the Women's Health Initiative Observational Study proved that the E-selectin levels could be considered as a predictor of diabetes among the USA women.¹⁰⁷ The decreased adiponectin levels among the obese T2DM patients could be due to the decreased adiponectin production by the enlarged adipocytes in the states of increased adiposity, since adiponectin is predominantly secreted by the pre-adipocytes.¹⁰⁸ One of the mechanisms for the negative correlation between the adiposity and adiponectin levels might be the increased secretion of TNF- α (TNF- α) from the accumulated visceral fat which potentially inhibits adiponectin secretion.^{109–111}

A study conducted by Vaverkova et al. independently found a positive association of soluble vCAM-1 with adiponectin.¹¹² In another study of the high risk dyslipidemic patients, these authors found altered levels of these biomarkers in the patients with vascular disease or dyslipidemia.¹¹³ The existence of the positive association between the soluble vCAM-1 and adiponectin was also recently described in T2DM patients with diabetic nephropathy and was associated with the endothelial dysfunction measured by the flow-mediated dilatation.¹¹⁴

The association between the insulin resistance and the endothelial dysfunction can be explained by the decreased dihydropterin reductase activity caused by the insulin resistance with the subsequent depletion of an essential cofactor of the catalytic activity of nitric oxide synthase (NOS), the tetrahydrobiopterin (BH4).¹¹⁵ This BH4 depletion might lead to the increased levels of the oxidative stress and endothelial dysfunction.¹¹⁵ In agreement with the observations that insulin can serve as a vasodilator and stimulate endothelial nitric oxide (NO) production,^{116,117} several studies have demonstrated that the T2DM patients might be characterized by the abnormal NO-mediated

vasodilation.¹¹⁸ In the same way as insulin resistance may contribute to the endothelial dysfunction, the defects in the NO-mediated vasodilation may contribute to the insulin resistance.¹¹⁹

Another possible mechanism of the endothelial dysfunction induced by insulin resistance is based on the impaired ability of insulin to inhibit very low density lipoprotein (VLDL) production in the liver of the T2DN patients.¹²⁰ An increase in serum triglycerides is accompanied by generation of small dense low density lipoprotein (LDL) particles that also contributes to the endothelial dysfunction in patients with type 2 diabetes.¹²¹

The association between the insulin resistance and the hypoadiponectinemia can be due to the effects of high blood levels of glucose and fatty acids. High blood levels of fatty acids are the direct cause of the insulin resistance. According to Dresner *et al.*¹²² and Griffin *et al.*,¹²³ an increase in the delivery of fatty acids to the muscles or a decrease in the intracellular metabolism of fatty acids might lead to the accumulation of the intracellular fatty acid metabolites, such as diacylglycerol, fatty acyl CoA, and ceramides. These metabolites activate a serine/threonine kinase cascade leading to the phosphorylation of serine and threonine sites of the insulin receptor substrate 1 (IRS1) and the insulin receptor substrate 2 (IRS2). This, in turn, reduces the ability of these IRS1 and IRS2 to activate phosphatidylinositol 3 kinase (PI 3 kinase) and eventually leads to the reduced activity of the glucose transporter 4 (GLUT4).^{122,123} As a consequence, the glucose uptake in the skeletal muscle cells is reduced because the diminished glucose transport activity of the insulin receptors. The decrease in the levels of plasma adiponectin can cause the decreased glucose uptake, increased gluconeogenesis, and decreased fatty acid oxidation in the skeletal muscles and the liver. The decrease in the oxidation of fatty acids defines the increase in the levels of free fatty acids, followed by the increase in the insulin resistance, and finally leading to the decrease in the glucose uptake.^{122,123} The decrease in the glucose uptake and the increase in the gluconeogenesis ultimately result in the increase in the levels of plasma glucose leading to T2DM.¹²⁴

Four biomarkers used in our study clearly belong to the class of multitasking proteins which often rely on intrinsic disorder for their multifunctionality. For example, adiponectin is a unique and abundant protein hormone that serves as an adipokine responsible

for the modulation of numerous metabolic processes, such as glucose regulation and fatty acid catabolism.^{125,126} At least four major biological functions were ascribed to adiponectin, regulation of metabolism, vascular protection, anti-inflammatory response, and cardioprotection/anti-ischemic function.^{126,127} In these functions, adiponectin regulates metabolism by participating in the increase in the insulin sensitivity, glucose utilization, and fatty acid oxidation. The vascular protective function is based on the adiponectin's roles in the enhancement of the NO production and the angiogenesis stimulation, whereas its anti-inflammatory role relies on the decrease in both neutrophil adhesion and macrophage activation.¹²⁷ Human adiponectin has 244 residues and includes a signal peptide (residues 1–18) that targets adiponectin for extracellular section and is cleaved in the mature protein, a non-conserved N-terminal region (residues 19–41) followed by the collagen-like domain (residues 42–107), and a C-terminal globular domain (residues 108–244). Structurally, this C-terminal globular domain of human adiponectin is similar to TNF- α , despite dissimilar amino acid sequences.¹²⁶ In agreement with the results of our computational analysis, biological activities of the full-length adiponectin are known to be controlled by numerous post-translational modifications (*e.g.*, hydroxylation and glycosylation). The adipocyte-secreted adiponectin exists in three forms, trimers (~90 kDa; the basic unit), low-molecular-weight hexamers (~180 kDa), and high-molecular-weight isoforms consisting of 12-mers to 18-mers (which can exceed 400 kDa).¹²⁶ Again, in agreement with our bioinformatics analysis, monomeric adiponectin is thermodynamically unstable and has not been observed under native conditions, whereas its proteolytic cleavage product containing globular C-terminal domain has been postulated to exist *in vivo*.¹²⁶ Curiously, the Arg112Cys and Ile164Thr mutations that prevent adiponectin from trimer formation and result in the impaired secretion of this protein from the cell were shown to be clinically associated with hypoadiponectinemia.⁸⁰

Intercellular cell adhesion molecule-1 (iCAM-1), also known as CD54 (cluster of differentiation 54), is an immunoglobulin-like glycoprotein expressed on the surface of several cell types including endothelial cells and cells involved in the immune response,¹²⁸ where it serves as a ligand for the leukocyte adhesion protein LFA-1 (integrin α -L/ β -2). Besides its major role in stabilizing cell-cell

interactions and facilitating leukocyte endothelial transmigration, iCAM-1 serves as a site for the cellular entry of human rhinovirus^{129,130} and is involved in a signal transduction¹³¹ and spermatogenesis.¹³² Human iCAM-1 has a signal peptide (residues 1–27) and is known to exist in the membrane-bound and soluble forms, with the transmembrane form possessing a large N-terminal extracellular domain (residues 28–480), a single-span transmembrane region (residues 481–503), and a small C-terminal cytoplasmic domain (residues 504–532). Human iCAM-1 is a heavily glycosylated and phosphorylated protein, with the extracellular domain being composed of multiple loops stabilized by seven disulfide bonds (Cys48–Cys92, Cys52–Cys96, Cys135–186, Cys237–290, Cys332–Cys371, Cys403–Cys419, and Cys431–Cys457). Curiously, many of these loops correspond to the regions predicted to be disordered in our study.

Vascular cell adhesion molecule-1 (vCAM-1), also known as cluster of differentiation 106 (CD106), is an important cell adhesion protein mediating the adhesion of the components of the immune system (such as lymphocytes, monocytes, eosinophils, and basophils) to vascular endothelium.^{133,134} It also functions in leukocyte-endothelial cell signal transduction, it may play a role in the development of atherosclerosis, and rheumatoid arthritis, and may be used by tumor cells to escape T-cell immunity.¹³⁴ Organization of vCAM-1 is very similar to that of iCAM-1, and human CD106 has a signal peptide (residues 1–24), a large N-terminal extracellular domain (residues 25–698), a single-span transmembrane region (residues 699–720), and a short C-terminal cytoplasmic domain (residues 721–739). The protein is glycosylated at multiple sites and has several disulfide bond-stabilized loops (Cys47–Cys95, Cys52–Cys99, Cys137–195, Cys246–291, Cys335–Cys383, and Cys534–Cys579). Similar to iCAM-1, many of these loops are predicted to contain significant amounts of disorder.

Finally, E-selectin, also known as cluster of differentiation 62 (CD62) antigen-like family member E (CD62E), endothelial-leukocyte adhesion molecule 1 (ELAM-1), or leukocyte-endothelial cell adhesion molecule 2 (LECAM2), is a cell adhesion protein expressed only by the cytokine-activated endothelial cells.¹³⁵ E-selectin plays a role in immunoadhesion by mediating the adhesion of blood neutrophils in cytokine-activated endothelium through interaction with PSGL1/SELPLG.¹³⁶ Since E-selectin is a typical adhesion, its topological structure, being similar to those of

iCAM-1 and vCAM-1, includes a signal peptide (residues 1–21), a large N-terminal extracellular domain (residues 22–556) that contains C-type lectin domain (residues 22–139), an EGF (epidermal-growth-factor)-like domain (residues 140–175), six Sushi domain (SCR repeat) units (residues 178–249, 240–301, 303–364, 366–427, 429–490, and 491–549), a single-span transmembrane region (residues 557–578), and a short C-terminal cytoplasmic tail (residues 579–610). There are several glycosylation and phosphorylation sites in human E-selectin, and this protein is heavily cross-linked by 16 disulfide bridges (Cys40–Cys138, Cys111–Cys130, Cys143–154, Cys148–163, Cys164–Cys174, Cys210–Cys237, Cys242–Cys286, Cys272–Cys299, Cys304–349, Cys335–362, Cys367–Cys412, Cys398–Cys425, Cys430–Cys475, Cys461–Cys488, Cys493–534, and Cys520–Cys547). Since E-selectin is predicted to be noticeably more disordered than the iCAM-1 and vCAM-1 (the content of the PONDR® VSL2-predicted disordered residues in E-selectin is 42.0%, whereas iCAM-1 and vCAM-1 contain 36.1% and 35.3% disordered residues, respectively), it is likely that the larger number of disulfide bonds and their more complex pattern are needed to keep the structure of this protein stable.

Conclusions

There is an association between insulin resistance and both hypoadiponectinemia and endothelial dysfunction, and proteins used as biomarkers to emphasize these connections are predicted to contain substantial amount of intrinsic disorder. The elevated disorder content in these important proteins might explain their exceptional multifunctionality.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Author contributions

O.H.A. wrote the manuscript and analyzed data. F.A.M. analyzed data and contributed to discussion. E.H.A. contributed to discussion and reviewed/edited manuscript. V.N.U. analyzed data, contributed to discussion, and wrote the manuscript.

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