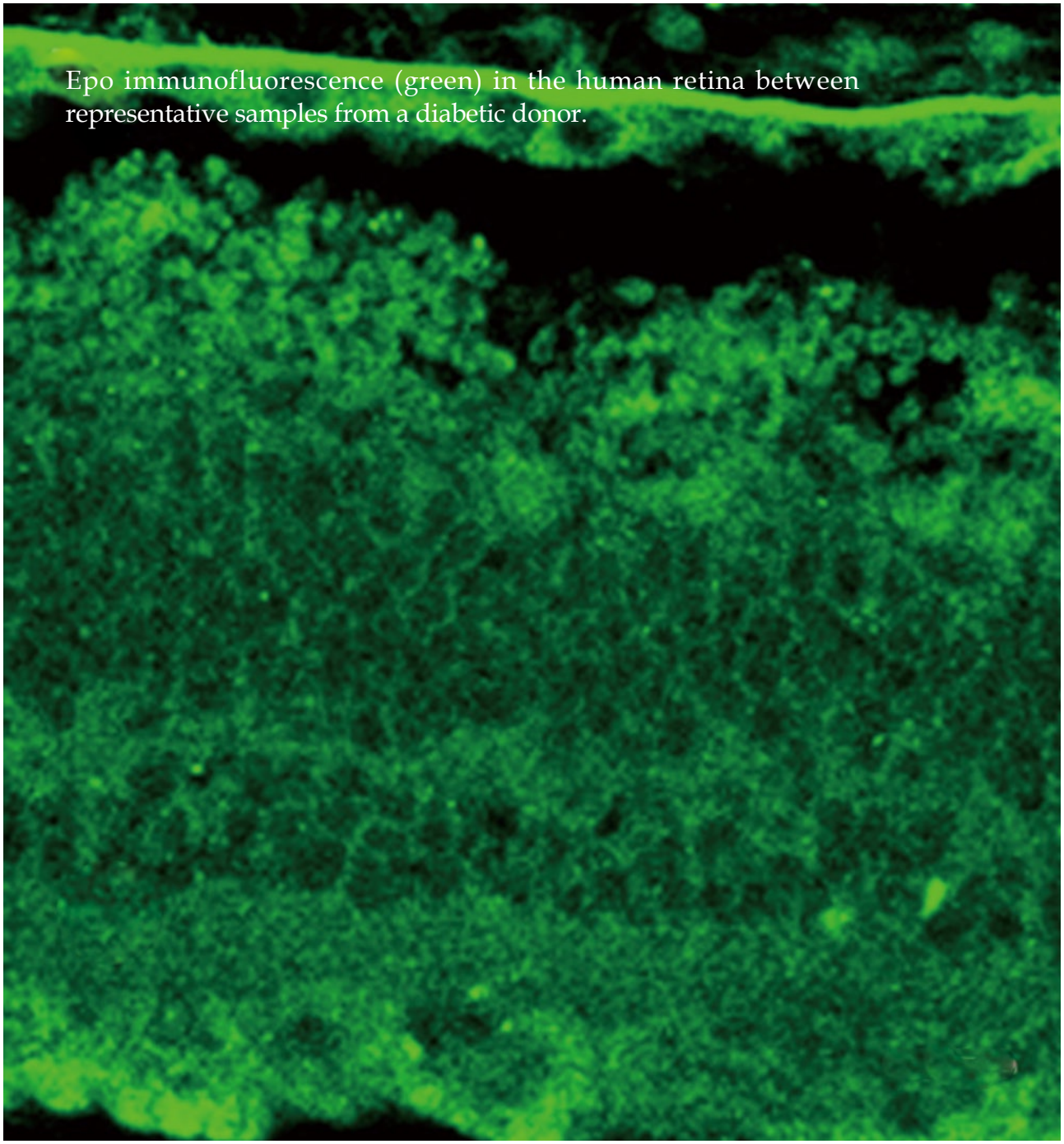
ISSN 1948-9358 (online)

World Journal of Diabetes

World J Diabetes 2010 May 15; 1(2): 27-64

A peer-reviewed, online, open-access journal of diabetes



Epo immunofluorescence (green) in the human retina between representative samples from a diabetic donor.

World Journal of Diabetes

A peer-reviewed, online, open-access journal of diabetes

Editorial Board

2010-2015

The *World Journal of Diabetes* Editorial Board consists of 323 members, representing a team of worldwide experts in diabetes mellitus. They are from 38 countries, including Argentina (1), Australia (13), Austria (6), Belgium (1), Brazil (3), Canada (14), China (21), Czech Republic (3), Denmark (9), Egypt (2), Finland (3), France (5), Germany (17), Greece (10), Hungary (2), India (10), Ireland (2), Iran (2), Israel (5), Italy (25), Japan (17), Malta (1), Netherlands (5), New Zealand (3), Oman (1), Poland (4), Romania (1), Singapore (2), South Korea (9), Spain (14), Sweden (3), Switzerland (1), Thailand (2), Turkey (9), United Arab Emirates (2), United Kingdom (11), United States (83), and Venezuela (1).

Michal Krcma, *Plzen*
Terezie Pelikanova, *Prague*

Denmark

Charlotte Brøns, *Gentofte*
Jens D Mikkelsen, *Copenhagen O*
Flemming Dela, *Copenhagen N*
Kristine Færch, *Gentofte*
R Scott Heller, *Gentofte*
Sandahl Christiansen, *Arhus C*
Filip K Knop, *Hellerup*
Esben T Vestergaard, *Aarhus N*
Milan Zdravkovic, *Søborg*

Egypt

Moshira AH Rateb, *Cairo*
Mona Farag Schaalan, *Cairo*

Finland

Gang Hu, *Helinski*
Qing Qiao, *Helsinki*
Karoliina Wehkalampi, *Helsinki*

France

Jean-Philippe Lavigne, *Nîmes Cedex*
Marie-Claude Morice, *Massy*
Gérard Said, *Paris*
Sophie Visvikis Siest, *Nancy*
Didier Vieau, *Villeneuve d'Ascq cédex*

Germany

Ioanna Gouni Berthold, *Cologne*
Roland Büttner, *Heidelberg*
Hammes Hans-Peter, *Mannheim*
Andrea Icks, *Düsseldorf*
Ulrich Arthur Julius, *Dresden*
Michael Kluge, *Munich*
Matthias Laudes, *Köln*
Ralf Lobmann, *Stuttgart*
Karsten Müssig, *Tübingen*
Rafael T Mikolajczyk, *Bremen*
Nahid Parvizi, *Neustadt a. Rbg*
Thomas Peter Reinehr, *Datteln*
Michael Ristow, *Jena*
Sven Schinner, *Duesseldorf*
Ovidiu A Stirban, *Bad Oeynhausen*
Silvia Anette Wein, *Kiel*
Christian Wrede, *Berlin*

Greece

Moses S Elisaf, *Ioannina*
Nikolaos Kadoglou, *Thessaloniki*
Gerasimos E Krassas, *Krini*
Demosthenes B Panagiotakos, *Athens*
Nikolaos Papanas, *Alexandroupolis*
Dimitrios Papazoglou, *Alexandroupolis*
Melpomeni Peppa, *Athens*
Nicholas K Tentolouris, *Athens*
Konstantinos Tziomalos, *Thessaloniki*
Elias Zintzaras, *Larissa*

Hungary

György Jermendy, *Maglodi*
Karoly Racz, *Szentkiralyi*

India

Sarika Arora, *New Delhi*
Subhabrata Chakrabarti, *Hyderabad*
Tapan K Chaudhuri, *New Delhi*
Kanwaljit Chopra, *Chandigarh*
Ravinder Goswami, *New Delhi*
SP Murthy, *Bangalore*
Viswanathan Mohan, *Chennai*
Anoop Misra, *New Delhi*
A Ramachandran, *Egmore Chennai*
Geetha Vani Rayasam, *Haryana*

Ireland

Amar Agha, *Dublin*
Mark Philip Hehir, *Dublin*

Iran

Mohammad Abdollahi, *Tehran*
Ahmad Esmaillzadeh, *Isfahan*

Israel

Shimon Efrat, *Tel Aviv*
Oren Froy, *Rehovot*
Eleazar Shafrir, *Jeusalem*
Haim Werner, *Tel Aviv*
Marina S Zimlichman, *Holon*

Italy

Antonio Aversa, *Rome*
Alessandro Bartolomucci, *Parma*
Giuseppina Basta, *Pisa*
Simona Bertoli, *Milano*
Fabio Broglio, *Torino*
Renzo Cordera, *Genova*
Maurizio Galderisi, *Naples*
Ezio Ghigo, *Turin*
Carla Giordano, *Palermo*
Riccarda Granata, *Turin*
Giorgio Iervasi, *Pisa*
Paolo Magni, *Milan*
Melania Manco, *Rome*
Piero Marchetti, *Pisa*
Lucia Pacifico, *Rome*
Stefano Palomba, *Catanzaro*
Giampaolo Papi, *Carpi*
Piermarco Piatti, *Milano*
Dario Pitocco, *Rome*
Manfredi Rizzo, *Palermo*
Raffaella Rosso, *Genoa*
Giuseppe Schillaci, *Perugia*
Giovanni Targher, *Verona*
Alberto Verrotti, *Chieti*
Andrea Viggiano, *Napoli*

Japan

Masato Asahina, *Chiba*
Takuya Awata, *Saitama-ken*
Satoshi Inoue, *Tokyo*
Takashi Kadowaki, *Tokyo*
Noriyuki Koibuchi, *Gunma*
Norikazu Maeda, *Osaka*
Kazuaki Nishio, *Tokyo*
Kenji Okumura, *Nagoya*
Toshiyasu Sasaoka, *Toyama*
Michio Shimabukuro, *Okinawa*
Kohzo Takebayashi, *Saitama*
Takashi Togo, *Yokohama*
Jun Udagawa, *Izumo*
Takuya Watanabe, *Tokyo*
Toshihiko Yada, *Tochigi*
Daisuke Yasuhara, *Kagoshima*
Tohru Yorifuji, *Kyoto*

Malta

Charles Savona Ventura, *Msida*

Netherlands

Sander Kersten, *Wageningen*
Edwin Mariman, *Maastricht*
Don Poldermans, *Rotterdam*
François Pouwer, *LE Tilburg*
Suat Simsek, *Alkmaar*

New Zealand

Paul Hofman, *Auckland*
Peter E Lobie, *Auckland*
Elaine Rush, *Auckland*

Oman

Jumana S Saleh, *Muscat*

Poland

Jerzy Beltowski, *Lublin*
Alicia H Dydejczyk, *Krakow*
Maciej Owecki, *Poznań*
Dorota Anna Zieba, *Krakow*

WJD World Journal of Diabetes

Contents

Bimonthly Volume 1 Number 2 May 15, 2010

Contents

World Journal of Diabetes
Volume 1 Number 2 May 15, 2010

AIM AND SCOPE

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a bimonthly, open-access, peer-reviewed journal supported by an editorial board of 323 experts in diabetes mellitus research from 38 countries.

The major task of *WJD* is to report rapidly the most recent results in basic and clinical research on diabetes including: metabolic syndrome, functions of α, β, δ and PP cells of the pancreatic islets, effect of insulin and insulin resistance, pancreatic islet transplantation, adipose cells and obesity, clinical trials, clinical diagnosis and treatment, rehabilitation, nursing and prevention. This covers epidemiology, etiology, immunology, pathology, genetics, genomics, proteomics, pharmacology, pharmacokinetics, pharmacogenetics, diagnosis and therapeutics. Reports on new techniques for treating diabetes are also welcome.

NAME OF JOURNAL
World Journal of Diabetes

LAUNCH DATE
March 15, 2010

SPONSOR
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: 0086-10-8538-1892
Fax: 0086-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

EDITING
Editorial Board of *World Journal of Diabetes*,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: 0086-10-5908-0038
Fax: 0086-10-8538-1893
E-mail: wjd@wjgnet.com
http://www.wjgnet.com

PUBLISHING
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: 0086-10-8538-1892
Fax: 0086-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

SUBSCRIPTION
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: 0086-10-8538-1892
Fax: 0086-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

ONLINE SUBSCRIPTION
One-Year Price 108.00 USD

PUBLICATION DATE
May 15, 2010

CSSN
ISSN 1948-9358 (online)

EDITORIAL OFFICE
Hai-Ning Zhang, Director
World Journal of Diabetes
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: 0086-10-5908-0038
Fax: 0086-10-8538-1893
E-mail: wjd@wjgnet.com
http://www.wjgnet.com

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1948-9358/g_info_20100107165233.htm. If you do not have web access please contact the editorial office.

ONLINE SUBMISSION
http://www.wjgnet.com/1948-9358office

World Journal of Diabetes

Online Submissions: http://www.wjgnet.com/1948-9358office
wjd@wjgnet.com
doi:10.4239/wjd.v1.i2.27

World J Diabetes 2010 May 15; 1(2): 27-35
ISSN 1948-9358 (online)

EDITORIAL

Exacerbation of chronic inflammatory diseases by infectious agents: Fact or fiction?

Cheng-Ming Wang, Bernhard Kaltenboeck

Cheng-Ming Wang, Ross University School of Veterinary Medicine, PO Box 334, Basseterre, St. Kitts, West Indies
Bernhard Kaltenboeck, Department of Pathobiology, College of Veterinary Medicine, Auburn University, 270 Greene Hall, Auburn, AL 36849-5519, United States
Author contributions: Wang CM and Kaltenboeck B structured the outline of this article; and Wang CM drafted this manuscript and both authors did the final editing.
Supported in part by NIH grant AI47202, and by grants from the Diabetes Trust Foundation, Diabetes Action Research and Education Foundation Grant #198, and by Graduate-in-Aid and Competitive Research Grant Programs at Auburn University
Correspondence to: Cheng-Ming Wang, DVM, MSc, PhD, Assistant Professor, Ross University School of Veterinary Medicine, PO Box 334, Basseterre, St. Kitts, West Indies. chwang@rossvet.edu.kn
Telephone: +869-4654161 Fax: +869-4651203
Received: November 20, 2009 Revised: March 27, 2010
Accepted: April 3, 2010
Published online: May 15, 2010

Abstract

Chronic inflammatory diseases caused by obesity represent critical public health concerns worldwide. In these diseases such as metabolic syndrome, diabetes and atherosclerosis, adipose tissue acts as an endocrine organ that releases large quantities of inflammatory mediators into circulation. Besides classically recognized effectors on the development of obesity and resultant conditions, infection has attracted attention as an enhancer of chronic inflammatory diseases. Infectious diseases have long been associated with obesity, metabolic syndrome, diabetes and atherosclerosis. However, the infectious hypothesis for chronic inflammatory diseases has been challenged by inconclusive clinical trials. Nevertheless, the large body of evidence accumulated over decades on the association of infectious diseases with obesity, diabetes and cardiovascular disease should not be disregarded. Instead, re-formulation of hypotheses of the mechanisms by which microbes affect obesity-associated diseases may be required with an emphasis on the early events in the progression of such diseases and the multifactorial nature of pathogen-host interactions. This review focuses on pathogens that directly promote obesity and on pathogens that cause chronic infections and thereby enhance metabolic diseases in obese patients. A new perspective on the interaction between infections and obesity-related diseases may improve management of chronic inflammatory diseases that rank high among global threats to human health.

Key words: Chronic inflammatory diseases; Obesity; Diabetes; Adenovirus-36; *Chlamydia pneumoniae*

Peer reviewers: Moshira Abdel Hakim Rateb, MD, Assistant Professor of Human Physiology, Faculty of Medicine (Kasr Al Aini.), Cairo University, Manial El Roda, Cairo, Egypt; Edwin Mariman, PhD, Professor, Department of Human Biology, Maastricht University, 6200 MD Maastricht, The Netherlands

Wang CM, Kaltenboeck B. Exacerbation of chronic inflammatory diseases by infectious agents: Fact or fiction? *World J Diabetes* 2010; 1(2): 27-35 Available from: URL: http://www.wjgnet.com/1948-9358/full/v1/i2/27.htm DOI: http://dx.doi.org/10.4239/wjd.v1.i2.27

INTRODUCTION

The incidence of obesity has dramatically increased during the recent decades worldwide. Currently, two-thirds of adults in the USA are overweight and around 32% are obese with obesity still trending upwards[1,2]. Worldwide, over 1 billion adults are overweight and more than 300 million are clinically obese (body mass index $\geqslant$ 30 kg/m^2)[3]. Alarmingly, obesity has also increased markedly in children[2]. It has only been recognized over

the last 15 years that obesity is an endocrine disease in which particularly white abdominal adipose tissue secretes large amounts of inflammatory mediators[4]. The chronic release of these mediators called adipocytokines in patients with high body mass index results in a combination of clinical symptoms characterized by high blood pressure, resistance to intracellular uptake of glucose such that glucose homeostasis requires increased insulin secretion (insulin resistance) and perturbation of the blood lipid profile (high total cholesterol and low-density lipoprotein). The cluster of these clinical symptoms is termed metabolic syndrome.

Obesity and its associated conditions such as metabolic syndrome, diabetes and atherosclerosis are now considered chronic inflammatory diseases[5]. Inflammation is the key characteristic of obesity and metabolic syndrome and production of pro-inflammatory cytokines such as TNF-α is essential to enhance the development of type 2 diabetes and atherosclerosis. For example, TNF-α induces insulin resistance by stimulating stress hormone production and decreasing tyrosine phosphorylation of insulin-induced insulin receptor substrate 1[6]. Similarly, inflammation is also a key characteristic of the host response to infectious agents[7].

While inflammation is a shared and key characteristic of both chronic inflammatory diseases and infections, infectious diseases have long been associated directly with obesity [i.e. Canine distemper virus (CDV), Rous-associated virus-7 (RAV-7), Borna disease virus (BDV), Scrapie agent and adenoviruses SMAM-1 and 36] as well as the consequences of obesity such as metabolic syndrome, diabetes and atherosclerosis [i.e. *Helicobacter pylori* (*H. pylori*), *Chlamydia pneumoniae* (*C. pneumoniae*), *Porphyromonas gingivalis* (*P. gingivalis*), hepatitis C virus (HCV) and human immunodeficiency virus (HIV)]. Clinical experience clearly shows that infectious diseases worsen glycemic control in diabetic patients[8-10]. Conversely, diabetic patients are also known to be at increased risk for infectious diseases and for higher severity of such diseases[8,11]. Obesity and exposure to infectious agents overlap in large population segments and therefore may mutually influence each other. Thus, profound practical medical benefits may result from a rational comprehensive approach to the management of chronic inflammatory diseases if therapy of either metabolic syndrome or infectious disease would also mitigate the respective other conditions.

This review focuses first on pathogens that directly promote obesity and, as a downstream consequence of obesity, the development of metabolic syndrome and its associated conditions. Subsequently, the review will focus on a second set of pathogens that cause chronic infections and subsequent release of inflammatory mediators and, *via* this mechanism, induce or exacerbate metabolic syndrome. A new perspective on the connection between infections and obesity-related diseases will better the management of these chronic inflammatory diseases that now rank very highly among the global threats to human health.

OBESITY-ENHANCING PATHOGENS

CDV

CDV is a lymphotropic and neurotropic negative-stranded RNA virus belonging to the genus *Morbillivirus*[12,13]. It affects mainly dogs and related mammals by invading the nervous system and replicating in neurons and glial cells of the white cell subgroup resulting in a frequently fatal disease[13]. Even though CDV is not considered a human pathogen, a suggestive association with human disease has been described[14]. CDV is antigenically closely related to human measles virus with both of them belonging to the same family of *Paramyxoviridae* viruses.

CDV was reported as the first obesity-promoting pathogen in 1982 when Lyons *et al*[15] published the landmark article in *Science*, "A virally induced obesity syndrome in mice" that reported that CDV infection induced obesity in Swiss Albino mice. CDV-inoculated mice showed increased body weight as well as an increased number and size of fat cells[15]. Anatomical damage and altered neurochemistry in the hypothalamus was subsequently demonstrated in CDV-infected mice[16-21]. The hypothalamus plays a well-documented role in appetite regulation, energy consumption and neuroendocrine function[18]. CDV-infected mice showed down-regulated leptin receptors in the hypothalamic area of the brain, explaining their inability to generate a proper response to leptin in the brain[18]. With lower number of leptin receptors, hunger may be induced despite high leptin plasma levels that signal satiety. In addition, CDV also down-regulates melanin-concentrating hormone[21], expression levels of neuropeptides and catecholamine[15,22] and production of proinflammatory cytokines[20]. Collectively, these data suggest that persistent CDV infection of the hypothalamus specifically alters satiety-signaling pathways and thereby induces excessive food consumption and eventually obesity.

RAV-7

RAV-7, an avian leukosis virus, was the second microbe reported to induce obesity. RAV-7 (avian leukosis virus subgroup C) is the most common poultry retrovirus associated with neoplastic disease[23]. RAV-7 causes obesity in chickens combined with growth stunting, hypertriglycemia, hypercholesterolemia as well as enlarged fatty liver, anemia and immunosuppression[23]. The lipid content of the diet did not influence the RAV-7-mediated induction of obesity[24]. By 20 d after hatching, infected chickens were smaller than uninfected hatch mates and developed ataxia and obesity over the next 30 d. These chickens also developed mild anemia and lipemia and had high levels of plasma uric acid. RAV-7 infection also induced a marked decrease in the weight of thymus and bursa of Fabricius[23]. The histological appearance of obesity is characterized in the liver by a diffuse panlobular accumulation of fat in microdroplets and by a lymphoblastoid cellular infiltrate in thyroid gland and pancreas. Carter *et al*[24] (1983) hypothesized that RAV-7 infection induces obesity by reducing thyroid hormone levels.

Carter *et al*[25] also investigated the specificity of obesity induction by RAV-7. Avian leucosis viruses of the subgroups A [RAV-1 and MAV-1 (O) causing osteopetrosis], B [MAV-2 (O) and MAV-2 (N) causing nephroblastoma], D (RAV-50) and F (RAV-61 and ring-necked pheasant virus) did not induce obesity[25].

BDV

Borna disease virus is an enveloped, non-segmented, negative-stranded RNA virus of the order *Mononegavirales* with replication and transcription inside the nucleus of the host cells[26-28]. BDV infection is found worldwide and induces fatal disease in numerous animals such as horse, sheep, cat and dog[29,30] and there is also evidence that BDV may affect humans[31,32]. Chalmers *et al*[32] (2005) reported between 0% to 48% BDV seropositivity and 0% to 82% BDV antigen prevalence in humans. Narayan *et al*[33] and Gosztonyi *et al*[34,35] described an obesity syndrome in rats apparently induced by BDV in an age-, genetic background- and virus strain-dependent manner. Infected obese rats showed massive visceral fat deposition with elevated serum glucose levels and hypertriglyceri demia[34]. Several investigators hypothesized that BDV infection induces obesity through inflammatory lesions and viral antigen expression in the brain, particularly in the hypothalamus, similar to CDV infection[36].

Scrapie agent

The causative agent of scrapie is thought to be a prion[37]. Scrapie agent causes a fatal neurodegenerative disease with a long incubation period in sheep and goats[38]. Scrapie agent is not known to infect humans. In the laboratory, many other animals such as hamster, mice, rats, voles, gerbils, mink, cattle and monkeys have been successfully infected with scrapie agent[37]. This disease is classified as transmissible spongiform encephalopathy, similar to human Creutzfeld-Jacob's disease and related TSEs caused by prions[39-40].

Kim *et al*[41] reported that the ME-7 scrapie strain induced obesity and vacuolization in the forebrain of mice but other strains did not. Since adrenalectomy prevented obesity, it was suggested that this scrapie agent induces obesity *via* the hypothalamic-pituitary-adrenal axis[42]. Scrapie agent was also confirmed to cause hyperglycemia, hyperinsulinemia and diabetes by inducing pancreatic lesions and a significant decrease of the glucose transporter GLUT-1 in the brain[43,44].

Adenovirus-SMAM-1

Adenoviruses are non-enveloped DNA viruses with icosahedral symmetry and a diameter of 65-80 nm[45]. Adenoviruses were first isolated in 1953 during establishment of cell lines from pediatric adenoidal tissues obtained by tonsillectomy[46]. Adenoviruses infect a wide range of hosts such as birds, mammals and humans. Approximately 8% of the world-wide reported virus infections were caused by adenoviruses which can cause serious respiratory disease of epidemic proportions reported with a group of military recruits[47]. There are 5 major subgroups of human adenoviruses and each subgroup is also subdivided into several serotypes. The viral genome consists of 5 early transcription units (E1A, E1B, E2, E3, and E4), 2 delayed early units (IX and Iva2) and one major late unit to generate mRNAs (L1-L5)[38]. Adenoviruses produce a variety of serious diseases in people of all ages.

SMAM-1 is a strain of avian adenovirus responsible for a poultry epidemic in India during the 1980s[48] and is serologically related to another poultry adenovirus, chick embryo lethal orphan virus (CELO). SMAM-1-inoculated 3 wk-old chickens showed 53% greater visceral fat 3 wk post inoculation compared to uninfected controls. Paradoxically, the increased adiposity in SMAM-1 infected chickens was accompanied by reduced body weight and lower blood concentrations of cholesterol and triglycerides than in controls[49]. Livers of the SMAM-1 infected chickens were significantly heavier and showed severe congestion, fatty infiltration and presence of intranuclear inclusion bodies under histopathological examination. The infected chickens showed also atrophied bursae, spleen and thymus[49]. Dhurandhar *et al*[50] (1997) reported an association between SMAM-1 seropositivity and human obesity. SMAM-1 antibody-positive humans showed significantly higher BMI and significantly lower blood cholesterol and triglycerides compared to the antibody-negative subjects.

Adenovirus-36

The Adenovirus-36 strain of adenoviruses was first identified in 1978 in Germany from the feces of a 6-year-old girl with diabetes and enteritis[51]. Accumulated evidence from animal models, *in vitro* experiments and human epidemiology strongly suggest a positive association between adenovirus-36 and human obesity. Atkinson et al showed that 11%-30% of Americans are seropositive against adenovirus-36[52]. Dhurandhar *et al*[53] (2000) explored the influence of adenovirus-36 infection on the development of obesity in chickens. Adenovirus-36 challenged chickens showed 100% greater visceral fat and total body weight than the control group inoculated with sterile cell culture medium and these conclusions were confirmed by the subsequent studies[54]. Adenovirus-36 inoculated male marmoset monkeys showed an astonishing 3-fold weight gain compared to uninfected controls[55]. *In vitro* studies showed that adenovirus-36 promotes the proliferation, differentiation and lipid accumulation in 3T3-L1 preadipocytes[38]. Atkinson et al screened the sera of 360 obese (BMI $\geqslant$ 30 kg/m^2) and 142 non obese (BMI $\leqslant$ 30 kg/m^2) subjects in Wisconsin, Florida and New York for adenovirus-36 antibodies. Adenovirus-36 antibodies were 30% and 11% prevalent in obese and non-obese subjects respectively and obese and non obese subjects with adenovirus-36 antibodies had significantly greater BMI than their respective seronegative counterparts[52]. The authors concluded that the influence of adenovirus-36 seropositivity on obesity

was highly significant independent of age, sex and origin of the human subjects[38,52]. *In vitro* studies shed light on the mechanisms of adenovirus-36-inducing obesity. Adenovirus-36 infected human preadipocytes showed increased replication, differentiation, lipid accumulation as well as reduced leptin secretion in fat cells[38,54,56]. This effect is specific for adenovirus-36 and is not observed with nonadipogenic adenovirus (Ad-2). It is likely that adenovirus-36 infection increases the number of fat cells, glucose uptake by adipocytes and promotes lipogenesis[56]. Na *et al*[57] and Atkinson *et al*[58] reported a positive association between human adenovirus-36 and obesity in children. However, two recent epidemiological surveys[59,60] indicated that human adenovirus-36 did not play a direct role in the development of obesity in both Western Europe and the US.

Adenovirus type 5 has been widely used for gene therapy because it is a safe and efficient vector and accommodates large antigen-encoding structures[61]. So *et al*[62] (2005) showed that adenovirus-5 infected mice attained significantly greater body weight and higher adiposity than control group 22-23 wk post inoculation. The same mechanisms as found in adenovirus-36, i.e. increased preadipocyte differentiation, also apply for adenovirus-5 infection[56]. Human adenovirus-37 was first isolated by de Jong *et al*[63]. Adenovirus-37 caused adiposity in chickens and the visceral fat pads were three times heavier in adenovirus-37-inoculated chickens than in controls. Different from adenovirus-36, adenovirus-37 infections did not induce reduced concentrations of serum cholesterol. Among over 50 strains of adenoviruses among five subgroups maintained by ATCC, strains adenovirus-36 and adenovirus-37, adenovirus-5 are adipogenic and adenovirus-2 and adenovirus-31 are not adipogenic. The potential of other human adenoviruses on the development of obesity remains unknown.

PATHOGENS ENHANCING HUMAN CHRONIC INFLAMMATORY DISEASES AND OBESITY

H. pylori

H. pylori is a spiral-shaped gram-negative flagellated bacterium and causes highly prevalent chronic infections worldwide[64]. *H. pylori* is the etiology of diseases such as gastritis and stomach cancer but most *H. pylori* infections are "silent" and produce no clinical symptoms and in particular are asymptomatic in childhood. Worldwide, up to 10% of children and 80% of adults show laboratory evidence of *H. pylori* infection. Aydemir *et al*[65] (2005) provided the first association between chronic *H. pylori* infection and insulin resistance. The homeostasis model assessment of insulin resistance was significantly higher in 36 *H. pylori*-positive subjects than in 27 *H. pylori*-negative ones[65]. Epidemiological evidence also supports the association of *H. pylori* seropositivity with cardiovascular diseases and elevated parameters of metabolic syndrome[66,67].

C. pneumoniae

C. pneumoniae, an intracellular respiratory bacterium, causes acute or chronic bronchitis and pneumonia[68] and is responsible for 10% of the cases of community-acquired pneumonia and approximately 5% of cases of bronchitis and sinusitis in adults[68-70]. Many epidemiological surveys, experimental studies and clinical trials have provided strong evidence for the association between *C. pneumoniae* infection and metabolic syndrome, insulin resistance and cardiovascular disease[70-82]. The current concept of the influence of *C. pneumoniae* on atherosclerosis is that *C. pneumoniae*-infected macrophages traffic to secondary organs including arterial endothelium, induce persistent infection and lead to the local upregulation of proinflammatory molecules (Figure 1A). Subsequently, infected macrophages and smooth muscle cells transform into foam cells and result in plaque destabilization, thrombus formation and myocardial infarction in arterial endothelium[75,85]. Ased on this notion, antibiotic treatment would reduce cardiovascular events by eliminating *C. pneumoniae* persistent infection and preventing re-infection. However, clinical trials with anti–biotic treatment based on this concept failed. This failure to reduce cardiovascular events by antibiotic treatment requires the reformulation of the current mechanistic understanding of the association between *C. pneumoniae* and metabolic syndrome and cardiovascular disease[83].

Wang *et al*[84] examined the influence of *C. pneumoniae* infection on progression of insulin resistance in dependence of host genetic background and dietary fat concentration in an obese mouse model. They concluded that murine *C. pneumoniae* infection enhances insulin resistance and diabetes in a genetically and nutritionally restricted manner *via* circulating inflammatory mediators such as TNF-α[84] and proposed a new mechanism of *C. pneumoniae*-induced exacerbation of insulin resistance developed in this investigation (Figure 1B). By quantifying the levels of *C. pneumoniae* and TNF-α transcripts in different organs, they concluded that the dispersal of a small number of *C. pneumoniae* organisms to secondary tissues was irrelevant to progression of insulin resistance and the early onset of type 2 diabetes. In contrast, the bulk infection of the lung caused an increase in circulating cytokines that drove the long-term exacerbation of insulin resistance (Figure 1B) and accelerated the onset of type 2 diabetes[84]. It was reported that combined pathogen burden[82] and positive serology for both *H. pylori* and *C. pneumoniae*[66] showed the strongest association with insulin resistance. These data suggest that exposure to multiple pathogens may potentiate chronic low-grade inflammation and insulin resistance and that the mechanisms whereby the pathogens affect chronic inflammatory diseases are shared.

P. gingivalis

A national survey with 9 689 subjects from 1988 to 1994 showed that periodontal disease is prevalent in the U.S. adult population[86]. Approximately 35% of the adults

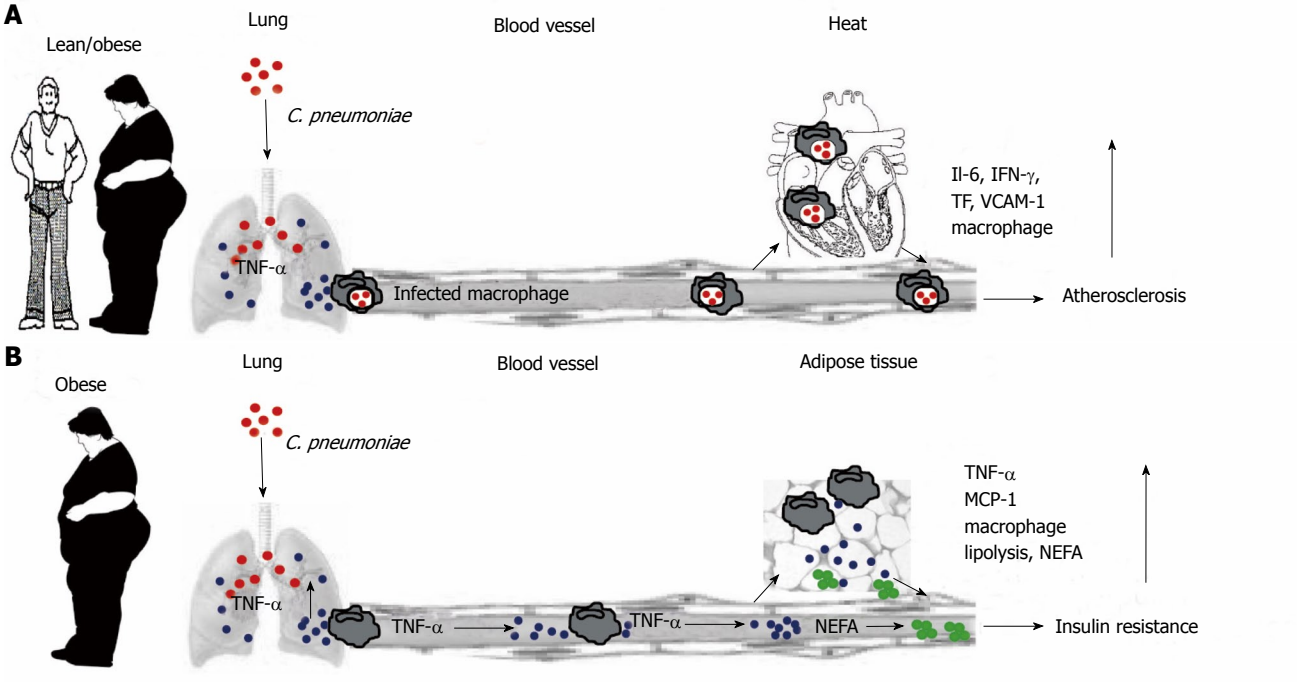


Figure 1 Schematic representation of *C. pneumoniae*-mediated acceleration of inflammatory diseases associated with metabolic syndrome. A: Current concept of the influence of *C. pneumoniae* on atherosclerosis. After respiratory infection, *C. pneumoniae* (red) is endocytosed by alveolar macrophages of infected lean or obese individuals. Infected macrophages re-enter circulation and traffic to secondary organs including arterial endothelium. Disseminated *C. pneumoniae* organisms infect other cell types, leading to up-regulation of pro-inflammatory molecules such as IL-6, IFN-γ, tissue factor and vascular-cell-adhesion molecule 1. These cytokines, in particular IFN-γ, retard chlamydial replication and induce persistent infection. In arterial endothelium, infected macrophages and smooth muscle cells transform into foam cells, affecting atheroma biology and leading to plaque destabilization, thrombus formation or myocardial infarction[85]. B: Concept of *C. pneumoniae*-induced exacerbation of insulin resistance developed by Wang *et al*[84]. *C. pneumoniae* infection results in lung colonization, thereby increasing TNF-α release (blue). Circulating TNF-α induces insulin resistance by inhibiting the function of insulin receptor substrate-1 in peripheral tissues and further exacerbates insulin resistance by promoting lipolysis and increased NEFA (green) production in adipose tissue. TNF-α and NEFA promote further macrophage infiltration and excess production of pro-inflammatory molecules in adipose tissue. In contrast to the atherosclerosis concept, the perpetuation of adipose tissue inflammation is driven by circulating pro-inflammatory cytokines rather than by in situ production stimulated by infected macrophages. Sustained by continuous high-fat nutrition, the inflammatory condition of adipose tissue maintains the vicious cycle of insulin resistance in the absence of *C. pneumoniae* organisms.

in the USA have periodontitis, an inflammation that involves the periodontal ligament and alveolar bone, while about 75% have gingivitis, an inflammation of the gingival tissues surrounding the teeth[8,86]. Periodontal diseases are initiated by gram-negative and anaerobic bacteria such as *P. gingivalis* residing in biofilms on gingival tissues and teeth[87]. Periodontal diseases had been thought of as localized conditions of concern only to dental health professionals. Emerging evidence now suggests that periodontal diseases also exacerbate systemic conditions such as metabolic syndrome and diabetes[88,89]. The oral infection causes elevated circulating IL-1β and TNF-α which lead to hyperlipidemia and development of diabetes[88,90].

HCV

HCV infection is a worldwide problem with approximately 200 million infected individuals[1,2]. Chronic HCV infection may result in liver cirrhosis and hepatocellular carcinoma and is associated with multifaceted disease such as porphyria cutanea tarda, membranoproliferative glomerulonephritis and cryoglobulinemia. While epidemiological studies suggested a linkage between HCV infections and type 2 diabetes[91-95], Shintani *et al*[91] confirmed the direct involvement of HCV infection in the development of insulin resistance in a mouse model using mice with the HCV core gene inserted in their genome. They showed that a high level of TNF-α was the main factor to induce insulin resistance in HCV-transgenic mice and insulin sensitivity was restored by administration of anti-TNF-α antibody.

HIV

Insulin resistance is common in HIV-infected people and the prevalence of hyperglycemia and diabetes is significantly higher in people with HIV infection treated with antiretrovirals as compared with the general population[96]. The prevalence of insulin resistance in HIV subjects is around 35% and up to 47% when they received protease inhibitor therapy, while the incidence of insulin resistance is only around 5% in the general population[97]. Presumably, HIV induces an increased inflammatory state, as evident in elevated levels of adiponectin and free fatty acids in HIV-infected individuals[96].

CONCLUSION

Ranked as one of the three major challenges to human progress along with war and famine, infectious diseases remain among the leading causes of death and disability

Table 1 Pathogens associated with obesity

Pathogen	Effect	Mechanisms	Ref
CDV	Increased body weight in Swiss albino mice	Altered hypothalamic integrity; increased cytokine production, hyperinsulinemia and decreased leptin and neuropeptides	[15-17,21, 22]
RAV-7	Stunting, anemia and increased visceral fat in white leghorn chickens	Decreased thyroxine, hyperlipidemia and hyperinsulinemia	[23,24]
BDV	Obesity with increased visceral fat in Lewis rats	Inflammatory lesions in hypothalamus, increased triglyceride and blood glucose	[35,36]
SMAM-1	Stunting and increased visceral fat in white leghorn chickens	Impaired liver function and lipogenesis and glucagon deficiency	[49,50]
Scrapie agent	Increased body weight and fat accumulation in mice	Altered brain function and reduced GLUT-1	[43,44]
Adenovirus	Increased body weight in chickens, mice, rats and monkeys; seropositive subjects were heavier than seronegative counterparts	Increased replication, differentiation and lipid accumulation of preadipocytes	[51-60]

CDV: Canine distemper virus; RAV-7: Rous-associated virus-7; BDV: Borna disease virus; Ref: Reference.

Table 2 Pathogens associated with increased chronic inflammatory diseases in humans

Pathogen	Effect	Mechanisms	Ref
H. pylori	Affected subjects showed increased insulin resistance	Increased concentrations of plasma glucose and lipids	[64-67]
C. pneumoniae	Increased metabolic syndrome, insulin resistance and cardiovascular disease	Increased production of proinflammatory and circulating cytokines	[68-72]
P. gingivalis	Adults with dental infections demonstrated higher chance of insulin resistance and diabetes	Increased oxidative stress, advanced glycation end-products and altered immune function	[86-90]
HCV	Infected patients showed increased chance to develop insulin resistance and diabetes	Increased production of TNF and IL-6	[92-95]
HIV	HIV patients showed higher insulin resistance (35%) compared to normal subjects (5%)	Impaired glucose tolerance and significant hyperinsulinaemia	[96,97]

H. pylori: *Helicobacter pylori; C. pneumoniae*: *Chlamydia pneumoniae; P. gingivalis*: *Porphyromonas gingivalis;* HCV: Hepatitis C virus; HIV: human immunodeficiency virus; Ref: Reference.

worldwide[98]. More than 25% of annual human deaths are the direct result of infectious diseases[98,99]. Progress in diagnostic methodology now allows the consistent detection of low-number but widely-prevalent pathogens as well as sensitive and precise quantification that captures subtle elevations of inflammatory cytokines which are closely related to metabolism and the immune response. Application of these essential tools in epidemiological, pathological and experimental studies has strongly suggested an infectious influence on obesity, metabolic syndrome, diabetes and cardiovascular diseases.

Following Darwinian adaption in human evolution, thrifty genes have probably evolved to maximize food utilization during periods of mass starvation.This metabolic adaptation combines frugal use of nutrients with vigorous inflammatory responses to pathogens and thus maximizes survival[100,101]. While advantageous under selective pressure of starvation and epidemic infectious diseases, this genetic makeup of large population segments has become a liability in times of food abundance and increased hygiene that eliminates epidemic but not endemic chronic infections. The evolved frugal metabolic characteristics increase obesity under conditions of over-nutrition and intensive inflammatory responses enhance the pathological consequences of obesity under constant stimulation by previously unrecognized ubiquitous but low-level chronic infections.

In summary, the dominant influence on chronic inflammatory diseases is anchored in human genetics and the outcome is driven by food supply and consumption. In this cause-effect network with tightly integrated metabolic and immune response pathways, infections likely play a heretofore underappreciated role in modulating intensity and pathological consequences, thus potentially decisively modulating the cause-effect network in the pathogenesis of chronic inflammatory diseases. Confirmation of the infectious modulation of obesity (Table 1) and chronic inflammatory diseases (Table 2) will facilitate prevention and management of such diseases. Very likely, vaccinations against multiple infectious agents will be the sole effective and realistic approach to ameliorate pathogen-enhanced obesity and related conditions such as diabetes and atherosclerosis.

REFERENCES

1 **Ogden CL**, Carroll MD, Curtin LR, McDowell MA, Tabak CJ, Flegal KM. Prevalence of overweight and obesity in the United States, 1999-2004. *JAMA* 2006; **295**: 1549-1555

2 **Hotamisligil GS**, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 1993; **259**: 87-91

3 **Guilbert JJ**. The world health report 2002 - reducing risks, promoting healthy life. Educ *Health* (Abingdon) 2003; **16**: 230

4 **Weisberg SP**, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003; **112**: 1796-1808

5 **Galinier A**, Carrière A, Fernandez Y, Carpéné C, André M, Caspar-Bauguil S, Thouvenot JP, Périquet B, Pénicaud L, Casteilla L. Adipose tissue proadipogenic redox changes in obesity. *J Biol Chem* 2006; **281**: 12682-12687

6 **Feinstein R**, Kanety H, Papa MZ, Lunenfeld B, Karasik A. Tumor necrosis factor-alpha suppresses insulin-induced tyrosine phosphorylation of insulin receptor and its substrates. *J Biol Chem* 1993; **268**: 26055-26058
7 **Huang J**, DeGraves FJ, Lenz SD, Gao D, Feng P, Li D, Schlapp T, Kaltenboeck B. The quantity of nitric oxide released by macrophages regulates Chlamydia-induced disease. *Proc Natl Acad Sci USA* 2002; **99**: 3914-3919
8 **Mealey BL**, Rose LF. Diabetes mellitus and inflammatory periodontal diseases. *Compend Contin Educ Dent* 2008; **29**: 402-408, 410, 412-413
9 **Begue RE**, Mirza A, Compton T, Gomez R, Vargas A. Helicobacter pylori infection and insulin requirement among children with type 1 diabetes mellitus. *Pediatrics* 1999; **103**: e83
10 **Taylor GW**, Burt BA, Becker MP, Genco RJ, Shlossman M, Knowler WC, Pettitt DJ. Severe periodontitis and risk for poor glycemic control in patients with non-insulin-dependent diabetes mellitus. *J Periodontol* 1996; **67**: 1085-1093
11 **Bertoni AG**, Saydah S, Brancati FL. Diabetes and the risk of infection-related mortality in the U.S. *Diabetes Care* 2001; **24**: 1044-1049
12 **Rozenblatt S**, Eizenberg O, Englund G, Bellini WJ. Cloning and characterization of DNA complementary to the canine distemper virus mRNA encoding matrix, phosphoprotein, and nucleocapsid protein. *J Virol* 1985; **53**: 691-694
13 **Vandevelde M**, Zurbriggen A. The neurobiology of canine distemper virus infection. *Vet Microbiol* 1995; **44**: 271-280
14 **Summers BA**, Appel MJ. Aspects of canine distemper virus and measles virus encephalomyelitis. *Neuropathol Appl Neurobiol* 1994; **20**: 525-534
15 **Lyons MJ**, Faust IM, Hemmes RB, Buskirk DR, Hirsch J, Zabriskie JB. A virally induced obesity syndrome in mice. *Science* 1982; **216**: 82-85
16 **Bernard A**, Zwingelstein G, Meister R, Wild TF. Hyperinsulinemia induced by canine distemper virus infection of mice and its correlation with the appearance of obesity. *Comp Biochem Physiol B* 1988; **91**: 691-696
17 **Bernard A**, Fevre-Montange M, Giraudon P, Hardin H, Wild TF, Belin MF. [Demonstration of viral proteins and RNA in hypothalamus of mice infected by canine distemper virus]. *C R Acad Sci III* 1991; **313**: 545-551
18 **Bernard A**, Cohen R, Khuth ST, Vedrine B, Verlaeten O, Akaoka H, Giraudon P, Belin MF. Alteration of the leptin network in late morbid obesity induced in mice by brain infection with canine distemper virus. *J Virol* 1999; **73**: 7317-7327
19 **Nagashima K**, Zabriskie JB, Lyons MJ. Virus-induced obesity in mice: association with a hypothalamic lesion. *J Neuropathol Exp Neurol* 1992; **51**: 101-109
20 **Bencsik A**, Akaoka H, Giraudon P, Belin MF, Bernard A. Inhibition of tyrosine hydroxylase expression within the substantia nigra of mice infected with canine distemper virus. *J Neuropathol Exp Neurol* 1997; **56**: 673-685
21 **Verlaeten O**, Casery C, Cavagna S, Naville D, Giraudon P, Belin MF, Begeot M, Bernard A. Identification of Urop11, a novel leptin-modulated gene that is upregulated in the hypothalamus of mice with virus-induced obesity. *J Mol Endocrinol* 2007; **38**: 3-17
22 **Griffond B**, Verlaeten O, Belin MF, Risold PY, Bernard A. Specific alteration of the expression of selected hypothalamic neuropeptides during acute and late mouse brain infection using a morbillivirus: relevance to the late-onset obesity? *Brain Res* 2004; **1022**: 173-181
23 **Carter JK**, Ow CL, Smith RE. Rous-associated virus type 7 induces a syndrome in chickens characterized by stunting and obesity. *Infect Immun* 1983; **39**: 410-422
24 **Carter JK**, Garlich JD, Donaldson WE, Smith RE. Influence of diet on a retrovirus-induced obesity and stunting syndrome. *Avian Dis* 1983; **27**: 317-322
25 **Carter JK**, Smith RE. Specificity of avian leukosis virus-induced hyperlipidemia. *J Virol* 1984; **50**: 301-308
26 **Stitz L**, Dietzschold B, Carbone KM. Immunopathogenesis of Borna disease. *Curr Top Microbiol Immunol* 1995; **190**: 75-92
27 **Rott R**, Becht H. Natural and experimental Borna disease in animals. *Curr Top Microbiol Immunol* 1995; **190**: 17-30
28 **Kao M**, Hamir AN, Rupprecht CE, Fu ZF, Shankar V, Koprowski H, Dietzschold B. Detection of antibodies against Borna disease virus in sera and cerebrospinal fluid of horses in the USA. *Vet Rec* 1993; **132**: 241-244
29 **Ludwig H**, Bode L, Gosztonyi G. Borna disease: a persistent virus infection of the central nervous system. *Prog Med Virol* 1988; **35**: 107-151
30 **Richt JA**, VandeWoude S, Zink MC, Clements JE, Herzog S, Stitz L, Rott R, Narayan O. Infection with Borna disease virus: molecular and immunobiological characterization of the agent. *Clin Infect Dis* 1992; **14**: 1240-1250
31 **de la Torre JC**. Molecular biology of borna disease virus: prototype of a new group of animal viruses. *J Virol* 1994; **68**: 7669-7675
32 **Chalmers RM**, Thomas DR, Salmon RL. Borna disease virus and the evidence for human pathogenicity: a systematic review. *QJM* 2005; **98**: 255-274
33 **Narayan O**, Herzog S, Frese K, Scheefers H, Rott R. Behavioral disease in rats caused by immunopathological responses to persistent borna virus in the brain. *Science* 1983; **220**: 1401-1403
34 **Gosztonyi G**. Natural and experimental Borna disease virus infections--neuropathology and pathogenetic considerations. *APMIS Suppl* 2008; **124**: 53-57
35 **Gosztonyi G**, Ludwig H. Borna disease--neuropathology and pathogenesis. *Curr Top Microbiol Immunol* 1995; **190**: 39-73
36 **Herden C**, Herzog S, Richt JA, Nesseler A, Christ M, Failing K, Frese K. Distribution of Borna disease virus in the brain of rats infected with an obesity-inducing virus strain. *Brain Pathol* 2000; **10**: 39-48
37 **Atkinson RL**. Viruses as an etiology of obesity. *Mayo Clin Proc* 2007; **82**: 1192-1198
38 **Pasarica M**, Dhurandhar NV. Infectobesity: obesity of infectious origin. *Adv Food Nutr Res* 2007; **52**: 61-102
39 **Prusiner SB**, Scott M, Foster D, Pan KM, Groth D, Mirenda C, Torchia M, Yang SL, Serban D, Carlson GA. Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. *Cell* 1990; **63**: 673-686
40 **Pan KM**, Baldwin M, Nguyen J, Gasset M, Serban A, Groth D, Mehlhorn I, Huang Z, Fletterick RJ, Cohen FE. Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. *Proc Natl Acad Sci USA* 1993; **90**: 10962-10966
41 **Kim YS**, Carp RI, Callahan SM, Wisniewski HM. Scrapie-induced obesity in mice. *J Infect Dis* 1987; **156**: 402-405
42 **Kim YS**, Carp RI, Callahan SM, Wisniewski HM. Adrenal involvement in scrapie-induced obesity. *Proc Soc Exp Biol Med* 1988; **189**: 21-27
43 **Carp RI**, Kim YS, Callahan SM. Pancreatic lesions and hypoglycemia-hyperinsulinemia in scrapie-injected hamsters. *J Infect Dis* 1990; **161**: 462-466
44 **Vorbrodt AW**, Dobrogowska DH, Tarnawski M, Meeker HC, Carp RI. Quantitative immunogold study of glucose transporter (GLUT-1) in five brain regions of scrapie-infected mice showing obesity and reduced glucose tolerance. *Acta Neuropathol* 2001; **102**: 278-284
45 **Pereira HG**, Huebner RJ, Ginsberg HS, van der Veen J. A short description of the adenovirus group. *Virology* 1963; **20**: 613-620
46 **Shen Y**, Shenk TE. Viruses and apoptosis. *Curr Opin Genet Dev* 1995; **5**: 105-111
47 **Rubin BA**. Clinical picture and epidemiology of adenovirus infections (a review). *Acta Microbiol Hung* 1993; **40**: 303-323
48 **van Ginneken V**, Sitnyakowsky L, Jeffery JE. "Infectobesity:

viral infections (especially with human adenovirus-36: Ad-36) may be a cause of obesity. *Med Hypotheses* 2009; **72**: 383-388
49 **Dhurandhar NV**, Kulkarni P, Ajinkya SM, Sherikar A. Effect of adenovirus infection on adiposity in chicken. *Vet Microbiol* 1992; **31**: 101-107
50 **Dhurandhar NV**, Kulkarni PR, Ajinkya SM, Sherikar AA, Atkinson RL. Association of adenovirus infection with human obesity. *Obes Res* 1997; **5**: 464-469
51 **Wigand R**, Gelderblom H, Wadell G. New human adenovirus (candidate adenovirus 36), a novel member of subgroup D. *Arch Virol* 1980; **64**: 225-233
52 **Atkinson RL**, Dhurandhar NV, Allison DB, Bowen RL, Israel BA, Albu JB, Augustus AS. Human adenovirus-36 is associated with increased body weight and paradoxical reduction of serum lipids. *Int J Obes* (Lond) 2005; **29**: 281-286
53 **Dhurandhar NV**, Israel BA, Kolesar JM, Mayhew GF, Cook ME, Atkinson RL. Increased adiposity in animals due to a human virus. *Int J Obes Relat Metab Disord* 2000; **24**: 989-996
54 **Pasarica M**, Shin AC, Yu M, Ou Yang HM, Rathod M, Jen KL, MohanKumar S, MohanKumar PS, Markward N, Dhurandhar NV. Human adenovirus 36 induces adiposity, increases insulin sensitivity, and alters hypothalamic monoamines in rats. *Obesity* (Silver Spring) 2006; **14**: 1905-1913
55 **Dhurandhar NV**, Whigham LD, Abbott DH, Schultz-Darken NJ, Israel BA, Bradley SM, Kemnitz JW, Allison DB, Atkinson RL. Human adenovirus Ad-36 promotes weight gain in male rhesus and marmoset monkeys. *J Nutr* 2002; **132**: 3155-3160
56 **Vangipuram SD**, Sheele J, Atkinson RL, Holland TC, Dhurandhar NV. A human adenovirus enhances preadipocyte differentiation. *Obes Res* 2004; **12**: 770-777
57 **Na HN**, Hong YM, Kim J, Kim HK, Jo I, Nam JH. Association between human adenovirus-36 and lipid disorders in Korean schoolchildren. *Int J Obes* (Lond) 2010; **34**: 89-93
58 **Atkinson RL**, Lee I, Shin HJ, He J. Human adenovirus-36 antibody status is associated with obesity in children. *Int J Pediatr Obes* 2009; 1-4
59 **Broderick MP**, Hansen CJ, Irvine M, Metzgar D, Campbell K, Baker C, Russell KL. Adenovirus 36 seropositivity is strongly associated with race and gender, but not obesity, among US military personnel. *Int J Obes* (Lond) 2010; **34**: 302-308
60 **Goossens VJ**, Dejager SA, Grauls GE, Gielen M, Vlietinck RF, Derom CA, Loos RJ, Rensen SS, Buurman WA, Greve JW, van Baak MA, Wolffs PF, Bruggeman CA, Hoebe CJ. Lack of Evidence for the Role of Human Adenovirus-36 in Obesity in a European Cohort. *Obesity* (Silver Spring) 2010; In press
61 **Kanerva A**, Hemminki A. Adenoviruses for treatment of cancer. *Ann Med* 2005; **37**: 33-43
62 **So PW**, Herlihy AH, Bell JD. Adiposity induced by adenovirus 5 inoculation. *Int J Obes* (Lond) 2005; **29**: 603-606
63 **de Jong JC**, Wigand R, Wadell G, Keller D, Muzerie CJ, Wermenbol AG, Schaap GJ. Adenovirus 37: identification and characterization of a medically important new adenovirus type of subgroup D. *J Med Virol* 1981; **7**: 105-118
64 **Cave DR**. Transmission and epidemiology of Helicobacter pylori. *Am J Med* 1996; **100**: 12S-17S; discussion 17S-18S
65 **Aydemir S**, Bayraktaroglu T, Sert M, Sokmen C, Atmaca H, Mungan G, Gun BD, Borazan A, Ustundag Y. The effect of Helicobacter pylori on insulin resistance. *Dig Dis Sci* 2005; **50**: 2090-2093
66 **Ekesbo R**, Nilsson PM, Lindholm LH, Persson K, Wadström T. Combined seropositivity for H. pylori and C. pneumoniae is associated with age, obesity and social factors. *J Cardiovasc Risk* 2000; **7**: 191-195
67 **Longo-Mbenza B**, Nkondi Nsenga J, Vangu Ngoma D. Prevention of the metabolic syndrome insulin resistance and the atherosclerotic diseases in Africans infected by Helicobacter pylori infection and treated by antibiotics. *Int J Cardiol* 2007; **121**: 229-238
68 **Grayston JT**, Kuo CC, Wang SP, Altman J. A new Chlamydia psittaci strain, TWAR, isolated in acute respiratory tract infections. *N Engl J Med* 1986; **315**: 161-168
69 **Kleemola M**, Saikku P, Visakorpi R, Wang SP, Grayston JT. Epidemics of pneumonia caused by TWAR, a new Chlamydia organism, in military trainees in Finland. *J Infect Dis* 1988; **157**: 230-236
70 **Grayston JT**. Chlamydia pneumoniae, strain TWAR pneumonia. *Annu Rev Med* 1992; **43**: 317-323
71 **Kuo CC**, Jackson LA, Campbell LA, Grayston JT. Chlamydia pneumoniae (TWAR). *Clin Microbiol Rev* 1995; **8**: 451-461
72 **Saikku P**, Leinonen M, Mattila K, Ekman MR, Nieminen MS, Mäkelä PH, Huttunen JK, Valtonen V. Serological evidence of an association of a novel Chlamydia, TWAR, with chronic coronary heart disease and acute myocardial infarction. *Lancet* 1988; **2**: 983-986
73 **Boman J**, Söderberg S, Forsberg J, Birgander LS, Allard A, Persson K, Jidell E, Kumlin U, Juto P, Waldenström A, Wadell G. High prevalence of Chlamydia pneumoniae DNA in peripheral blood mononuclear cells in patients with cardiovascular disease and in middle-aged blood donors. *J Infect Dis* 1998; **178**: 274-277
74 **Gupta S**, Leatham EW, Carrington D, Mendall MA, Kaski JC, Camm AJ. Elevated Chlamydia pneumoniae antibodies, cardiovascular events, and azithromycin in male survivors of myocardial infarction. *Circulation* 1997; **96**: 404-407
75 **Campbell LA**, Moazed TC, Kuo CC, Grayston JT. Preclinical models for Chlamydia pneumoniae and cardiovascular disease: hypercholesterolemic mice. *Clin Microbiol Infect* 1998; **4** Suppl 4: S23-S32
76 **Ekesbo R**, Nilsson PM, Lindholm LH, Persson K, Wadström T. Combined seropositivity for H. pylori and C. pneumoniae is associated with age, obesity and social factors. *J Cardiovasc Risk* 2000; **7**: 191-195
77 **Smieja M**, Mahony J, Petrich A, Boman J, Chernesky M. Association of circulating Chlamydia pneumoniae DNA with cardiovascular disease: a systematic review. *BMC Infect Dis* 2002; **2**: 21
78 **Dart AM**, Martin JL, Kay S. Association between past infection with Chlamydia pneumoniae and body mass index, low-density lipoprotein particle size and fasting insulin. *Int J Obes Relat Metab Disord* 2002; **26**: 464-468
79 **Cannon CP**, Braunwald E, McCabe CH, Grayston JT, Muhlestein B, Giugliano RP, Cairns R, Skene AM. Antibiotic treatment of Chlamydia pneumoniae after acute coronary syndrome. *N Engl J Med* 2005; **352**: 1646-1654
80 **Grayston JT**, Kronmal RA, Jackson LA, Parisi AF, Muhlestein JB, Cohen JD, Rogers WJ, Crouse JR, Borrowdale SL, Schron E, Knirsch C. Azithromycin for the secondary prevention of coronary events. *N Engl J Med* 2005; **352**: 1637-1645
81 **Nabipour I**, Vahdat K, Jafari SM, Pazoki R, Sanjdideh Z. The association of metabolic syndrome and Chlamydia pneumoniae, Helicobacter pylori, cytomegalovirus, and herpes simplex virus type 1: the Persian Gulf Healthy Heart Study. *Cardiovasc Diabetol* 2006; **5**: 25
82 **Fernández-Real JM**, López-Bermejo A, Vendrell J, Ferri MJ, Recasens M, Ricart W. Burden of infection and insulin resistance in healthy middle-aged men. *Diabetes Care* 2006; **29**: 1058-1064
83 **Anderson JL**. Infection, antibiotics, and atherothrombosis--end of the road or new beginnings? *N Engl J Med* 2005; **352**: 1706-1709
84 **Wang C**, Gao D, Kaltenboeck B. Acute Chlamydia pneumoniae reinfection accelerates the development of insulin resistance and diabetes in obese C57BL/6 mice. *J Infect Dis* 2009; **200**: 279-287
85 **Campbell LA**, Kuo CC. Chlamydia pneumoniae--an infectious risk factor for atherosclerosis? *Nat Rev Microbiol* 2004; **2**: 23-32
86 **Albandar JM**, Brunelle JA, Kingman A. Destructive perio-

dontal disease in adults 30 years of age and older in the United States, 1988-1994. *J Periodontol* 1999; **70**: 13-29
87 **Paster BJ**, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA, Sahasrabudhe A, Dewhirst FE. Bacterial diversity in human subgingival plaque. *J Bacteriol* 2001; **183**: 3770-3783
88 **Iacopino AM**. Periodontitis and diabetes interrelationships: role of inflammation. *Ann Periodontol* 2001; **6**: 125-137
89 **Pontes Andersen CC**, Flyvbjerg A, Buschard K, Holmstrup P. Relationship between periodontitis and diabetes: lessons from rodent studies. *J Periodontol* 2007; **78**: 1264-1275
90 **Ritchie CS**. Mechanistic links between type 2 diabetes and periodontitis. *J Dent* 2009; **37**: S578- S579
91 **Shintani Y**, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, Moriya K, Koike K. Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004; **126**: 840-848
92 **Allison ME**, Wreghitt T, Palmer CR, Alexander GJ. Evidence for a link between hepatitis C virus infection and diabetes mellitus in a cirrhotic population. *J Hepatol* 1994; **21**: 1135-1139
93 **Caronia S**, Taylor K, Pagliaro L, Carr C, Palazzo U, Petrik J, O'Rahilly S, Shore S, Tom BD, Alexander GJ. Further evidence for an association between non-insulin-dependent diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 1999; **30**: 1059-1063
94 **Mason AL**, Lau JY, Hoang N, Qian K, Alexander GJ, Xu L, Guo L, Jacob S, Regenstein FG, Zimmerman R, Everhart JE, Wasserfall C, Maclaren NK, Perrillo RP. Association of diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 1999; **29**: 328-333
95 **Mehta SH**, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. *Ann Intern Med* 2000; **133**: 592-599
96 **Aboud M**, Elgalib A, Kulasegaram R, Peters B. Insulin resistance and HIV infection: a review. *Int J Clin Pract* 2007; **61**: 463-472
97 **Hadigan C**, Yawetz S, Thomas A, Havers F, Sax PE, Grinspoon S. Metabolic effects of rosiglitazone in HIV lipodystrophy: a randomized, controlled trial. *Ann Intern Med* 2004; **140**: 786-794
98 **Morens DM**, Folkers GK, Fauci AS. The challenge of emerging and re-emerging infectious diseases. *Nature* 2004; **430**: 242-249
99 World Health Organization. The World Health Report 2004. World Health Organization: Genève, 2004
100 **Hotamisligil GS**. Inflammation and metabolic disorders. *Nature* 2006; **444**: 860-867
101 **Roth J**. Evolutionary speculation about tuberculosis and the metabolic and inflammatory processes of obesity. *JAMA* 2009; **301**: 2586-2588

S- Editor Zhang HN **L- Editor** Roemmele A **E- Editor** Liu N

World Journal of Diabetes

Online Submissions: http://www.wjgnet.com/1948-9358office
wjd@wjgnet.com
doi:10.4239/wjd.v1.i2.36

World J Diabetes 2010 May 15; 1(2): 36-47
ISSN 1948-9358 (online)

TOPIC HIGHLIGHT

Sarika Arora, MD, Assistant Professor, *Series Editor*

Surrogate markers of insulin resistance: A review

Bhawna Singh, Alpana Saxena

Bhawna Singh, Department of Biochemistry, GB Pant Hospital, New Delhi 110002, India
Alpana Saxena, Department of Biochemistry, Maulana Azad Medical College & LN Hospital, New Delhi 110002, India
Author contributions: Singh B was responsible for drafting of the manuscript, study concept and design; and Saxena A for Critical revision of the manuscript, study concept and design.
Correspondence to: Bhawna Singh, Assistant Professor, Department of Biochemistry, GB Pant Hospital, New Delhi 110002, India. bhawna172@gmail.com
Telephone: +91-11-25280272
Received: January 18, 2010 Revised: April 29, 2010
Accepted: May 6, 2010
Published online: May 15, 2010

Abstract

Insulin resistance is a hallmark of obesity, diabetes, and cardiovascular diseases, and leads to many of the abnormalities associated with metabolic syndrome. Our understanding of insulin resistance has improved tremendously over the years, but certain aspects of its estimation still remain elusive to researchers and clinicians. The quantitative assessment of insulin sensitivity is not routinely used during biochemical investigations for diagnostic purposes, but the emerging importance of insulin resistance has led to its wider application research studies. Evaluation of a number of clinical states where insulin sensitivity is compromised calls for assessment of insulin resistance. Insulin resistance is increasingly being assessed in various disease conditions where it aids in examining their pathogenesis, etiology and consequences. The hyperinsulinemic euglycemic glucose clamp is the gold standard method for the determination of insulin sensitivity, but is impractical as it is labor- and time-intensive. A number of surrogate indices have therefore been employed to simplify and improve the determination of insulin resistance. The object of this review is to highlight various aspects and methodologies for current and upcoming measures of insulin sensitivity/resistance. In-depth knowledge of these markers will help in better understanding and exploitation of the condition.

Key words: Insulin resistance; Markers; Insulin; Homeostatis model assessment; Quantitative insulin sensitivity check index

Peer reviewers: Min Du, PhD, Associate Professor, Department of Animal Science, University of Wyoming, Laramie, WY 82071, United States; Ernest Akingunola Adeghate, PhD, Professor, Department of Anatomy, Faculty of Medicine & Health Sciences, UAE University, PO Box 17666, Al Ain, United Arab Emirates

Singh B, Saxena A. Surrogate markers of insulin resistance: A review. *World J Diabetes* 2010; 1(2): 36-47 Available from: URL: http://www.wjgnet.com/1948-9358/full/v1/i2/36.htm DOI: http://dx.doi.org/10.4239/wjd.v1.i2.36

INTRODUCTION

Insulin is a key regulator of glucose homeostasis. Insulin resistance is established by genetic and environmental factors. Insulin resistance (IR) leads to impaired glucose tolerance, and plays an important pathophysiological role in the development of diabetes[1]. In addition, IR leads to many of the metabolic abnormalities associated with metabolic syndrome/syndrome X. Patients with insulin resistance are likely to have impaired fasting plasma glucose levels, which in turn enhance the prevalence of more atherogenic, small dense low-density lipoprotein (LDL) particles. Central obesity and insulin resistance form the basis of the pathophysiology of dyslipidemia, lack of glucose tolerance, and the existence of chronic subclinical inflammation and hypertension in metabolic syndrome. IR has been described as a condition where a greater than normal amount of insulin is required to obtain a quantitatively normal response[2]. Measuring

insulin resistance has progressed from its role in the pathogenesis of diabetes, to an even more important role.

IR: PATHOGENESIS

The mechanism underlying IR involves a complex network of metabolism of glucose and fat, with the inflammatory cascade playing an important role. The important actions of insulin are anti-lipolysis in adipose tissue and stimulation of lipoprotein lipase[3]. Expanded adipose tissue mass associated with obesity mobilises free fatty acids (FFA) in circulation through the action of the cyclic-AMP dependent enzyme hormone sensitive lipase. FFA are also released through lipolysis of Triglyceride (TG)-rich lipoproteins in tissues by means of lipoprotein lipase[4]. In insulin-sensitive tissue, excessive fatty acids create insulin resistance by means of the added substrate availability and by modifying down- stream signalling[5]. When insulin resistance sets in, the increased lipolysis of stored TG in adipose tissue produces more fatty acids. The increased FFA concentration inhibits the anti-lipolytic action of insulin. The role of innate immunity and infection has also been postulated in the development of insulin resistance and can predict the development of diabetes mellitus type Ⅱ[6,7].

Insulin resistance, metabolic syndrome and atherosclerotic events share a common inflammatory basis. Presence of a low-grade systemic inflammation is the main mechanism that leads to impaired insulin action[8].

DISEASE CONDITIONS ASSOCIATED WITH IR

IR is an important clinical and biochemical determinant, not only of diabetes but also of many other clinical states. There is a need to evaluate insulin resistance, since it is an underlying mechanism and predictor of cardio-vascular diseases, diabetes, hypertension, obesity and other consequences of metabolic syndrome and impaired insulin sensitivity. In nondiabetic individuals, the initial presentation associated with insulin resistance is hyperinsulinemia, impaired glucose tolerance, dyslipidemia [hypertriglyceridemia and decreased high-density lipoprotein (HDL) cholesterol] and hypertension[9]. Insulin resistance contributes significantly to the pathophysiology of type 2 diabetes and is a hallmark of obesity, dyslipidemias, hypertension, and other components of the metabolic syndrome[10,11]. The association between insulin resistance and subclinical or clinical cardio-vascular disease in both nondiabetic[12-14] and diabetic subjects[15,16] has been observed.

Insulin resistance has been an area of interest in recent times, as it has effects on wide array of diseases. The pathophysiological conditions coupled with insulin resistance have persistently increased and include small dense LDL particles[17], augmented postprandial lipemia[18], enhanced renal sodium retention and high uric acid levels[19], dysfibrinolysis[20] increased resting heart rate[21] and polycystic ovarian syndrome[22]. In clinical practice, a family history of diabetes, a history of polycystic ovarian syndrome, gestational diabetes, impaired glucose metabolism, and obesity should be seen as a herald of the possibility of insulin resistance[23].

ESTIMATION OF IR/MEASUREMENT OF IR

A marker is a measurable variable found in an available biological sample or detected by tissue imaging, which can reflect the underlying disease pathophysiology, predict future events and indicate the response to treatment. Markers serve as sensitive detectors of early target organ damage[24]. Currently, validated risk-assessment tools do not satisfactorily account for the increased risk factors associated with metabolic syndrome[25]. Hence the need to identify markers of this syndrome is imperative.

Estimation of insulin resistance is being studied widely in humans. It is of great importance to develop animal models that are appropriate to the investigation of the epidemiology, pathophysiological mechanisms, outcomes of therapeutic interventions, and clinical courses of patients with insulin resistance. Insulin resistance is an established independent predictor of a range of disorders. Resistance to insulin sets in long before any disease signs start appearing. It is important to categorize and treat individuals with insulin resistance as early as possible, because hyperinsulinemia might remain undiagnosed for a long period, thereby increasing the risk of the development of other components of the syndrome, and consequent diseases. Prompt recognition and management of this metabolic syndrome offers important preventive measures[23].

In addition to maintaining whole body glucose homeostasis and promoting efficient glucose utilization[26], there are many other important physiological targets of insulin, including the brain, pancreatic β-cells, heart and vascular endothelium, that help to coordinate and couple metabolic and cardiovascular homeostasis under healthy conditions[26-29]. An accurate method for easily evaluating insulin sensitivity and following changes after therapeutic intervention is thus required.

NEED FOR SURROGATE MARKERS

Quantifying insulin sensitivity/resistance in humans and animal models is of great importance for basic science investigations and eventual use in clinical practice[30].

Among the tools to characterize IR and measure whole-body insulin action, the euglycemic hyperinsulinemic clamp technique is the direct method of estimation of IR. As this requires insulin infusion and repeated blood sampling, there is a need for simple, accessible measures for the evaluation of insulin sensitivity. Most large scale epidemiological studies merely correlate fasting insulin levels with the concerned outcome.

Table 1 Various methods to measure insulin resistance

S No	Method	Comments	Advantages	Disadvantages
1	Hyperinsulinemic euglycemic glucose clamp	Gold standard method for quantifying insulin sensitivity	Direct measure of insulin under steady-state conditions	Laborious, involves intra venous infusion of insulin, frequent blood sampling
2	Oral glucose tolerance test	Clinically used to detect glucose intolerance	Helps in estimating other surrogate indices	Useful for glucose tolerance but not for IR
3	Fasting insulin	Most practical method to measure IR	Detects insulin resistance before clinical disease appears	Lack of standardization of the insulin assay procedure
4	Glucose/insulin ratio (G/I ratio)	comparable to insulin sensitivity measured by the FSIVGTTT	Highly sensitive & specific for insulin sensitivity	Does not aptly reveal the physiology of insulin sensitivity
5	Insulinogenic index (IGI)	index of β-cell function δI (0-30 min)/δG (0-30 min)	Measure of first-phase insulin response to glucose challenge	Not broadly validated
6	Homeostasis model assessment	Assesses inherent β-cell function and insulin sensitivity *HOMA-IR* = (G × I)/22.5	Simple, minimally invasive, predicts fasting steady-state G and I levels	Insulin sensitivity in subjects treated with insulin needs further validation
7	Quantitative insulin sensitivity check index (QUICKI)	Mathematical transformation of FBG and insulin *QUICKI* = 1/[log (I μU/mL) + log(G mg/dL)]	Consistent, precise index of insulin sensitivity, minimally invasive	Normal range to be established for each laboratory due to significant inter laboratory variations in insulin assay
8	Minimal model analysis of frequently sampled intravenous glucose tolerance test	Indirect measure of insulin sensitivity/resistance	Analysis using the computer program MINMOD	Multiple blood sampling
9	Glucose insulin (GI) product	Index of whole-body insulin sensitivity		
10	Fasting insulin resistance index (FIRI)	(fasting G × fasting I)/25		

G: Glucose; I: Insulin; IR: Insulin resistance; FBG: Fasting blood glucose.

IR can be assessed by various means. Most of the methods employed are difficult to apply in clinical practice. Since compensatory hyperinsulinemia is highly correlated with IR[31], it has been observed that it may offer a better way to identify insulin-resistant patients than do measurements of glucose intolerance. On the other hand, analytic methods for insulin measurements are not standardized, thus making it hard to compare absolute values of plasma insulin concentrations from one laboratory to another[32].

There has been an urgent need for the consideration of other parameters that can be used to assess IR, along with the development of novel surrogate markers of insulin resistance, which are more applicable for large population-based epidemiological investigations. Numerous such markers have been proposed on many occasions in the literature[33-39].

More than 15 years ago, the mathematical model of the normal physiological dynamics of insulin and glucose produced the homeostasis model assessment (HOMA), which provided equations for estimating insulin resistance (HOMA-IR) and β-cell function from simultaneous fasting measures of insulin and glucose levels[36]. AIn addition, the quantitative insulin sensitivity check index (QUICKI) derived from logarithmically-transformed fasting plasma glucose (FPG) and insulin levels has proven to be a first-rate index of insulin resistance in comparison with clamp-IR[40].

The efficacy and implication of surrogate assessment of insulin resistance depends on the extent to which it correlates with the direct estimate of this variable. Various methods to quantify insulin resistance have been described, and are shown below in Table 1.

Hyperinsulinemic euglycemic glucose clamp

The hyperinsulinemic euglycemic glucose clamp technique has been described as the gold standard method for quantifying insulin sensitivity[41]. It is the reference method for quantifying insulin sensitivity in humans because it directly measures the effects of insulin in promoting glucose utilization under steady-state conditions *in vivo*[41]. Direct estimation of IR by means of the euglycemic clamp technique and insulin suppression test (IST) is experimentally demanding, complicated, and impractical when large scale epidemiological studies are involved. These methods are laborious, painstaking and expensive, are therefore rarely used in larger-scale clinical research and, as such, are irrelevant for clinical practice. Consequently, over the years, a number of surrogate indices for insulin sensitivity or insulin resistance have been developed.

The glucose clamp is difficult to apply in large scale investigations because of the chaotic procedure, which involves intra-venous infusion of insulin, taking frequent blood samples over a 3 h period, and the continuous adjustment of a glucose infusion.

SURROGATE MARKERS

Oral glucose tolerance test

The oral glucose tolerance test (OGTT) is an easy test,

and is commonly used in medical practice to detect glucose intolerance as well as type 2 diabetes[42]. It involves the administration of glucose to find out how rapidly it is cleared from the blood stream. It implicates the efficiency of the body to utilize glucose after glucose load.

During OGTT, after 8 to 10 h of fasting, blood glucose levels are determined at 0, 30, 60, and 120 min following a standard oral glucose load (75 g)[42,43].

It imitates the normal physiology of the glucose and insulin flux more closely than conditions of the other methods such as the glucose clamp, IST, or the Frequently Sampled Intravenous Glucose Tolerance Test (FSIVGTT). Since glucose tolerance and insulin sensitivity are dissimilar conceptually, OGTT provides useful information about glucose tolerance but not insulin resistance. However, OGTT is also used to estimate other surrogate indices of insulin resistance. Impaired glucose tolerance offers few aberrations during OGTT. Firstly, rapid and continuous rise in plasma glucose concentration, and secondly, lack of decline below 140 mg/dL in plasma glucose at 2 h after attaining peak value. Subjects with impaired fasting glucose (IFG) have higher FPG than individuals with normal glucose tolerance or impaired glucose tolerance (IGT)[44].

Fasting insulin

Measurement of the fasting insulin level has long been considered the most practical approach for the measurement of insulin resistance[33]. It correlates well with insulin resistance. A considerable correlation has been found between fasting insulin levels and insulin action as measured by the clamp technique. A substantial overlap between insulin-resistant and normal subjects is a constraint, as there is a lack of standardization of the insulin assay procedure. Nevertheless, with a reliable insulin assay, insulin resistance can be detected early, before clinical disease appears[45].

As glucose levels change rapidly in the postprandial state, the use of fasting insulin for estimating IR should be done after an overnight fast, since the variable levels of glucose confound the simultaneous measure of insulin.

In healthy subjects, increased fasting insulin levels (with normal fasting glucose levels) correspond to insulin resistance. In this population 1/(fasting insulin) can substituted for insulin sensitivity that decreases as subjects become more insulin resistant (and fasting insulin levels rise)[33]. However, it does not cover the inappropriately low insulin secretion in the face of hyperglycemia seen in diabetic subjects or glucose-intolerant subjects.

Use of fasting insulin levels for assessment of IR is limited because of a high proportion of false-positive results and by lack of standardization. To overcome this issue, standardization of insulin assay has been recommended by the ADA Task Force, to be certified by a central laboratory[44].

A high plasma insulin value in individuals with normal glucose tolerance reflects insulin resistance, and high insulin levels presage the development of diabetes[45].

Glucose/insulin ratio

The Glucose/insulin (G/I) ratio has been employed in a number of studies as an index of insulin resistance[34,46,47]. Functionally, it will be equivalent to 1/(fasting insulin) in non- diabetics as fasting glucose levels are all in the normal range, though it does not appropriately reflect the physiology underlying the determinants of insulin sensitivity[48]. The fasting G/I ratio is a theoretically imperfect index of insulin sensitivity.

In a study conducted by Legro *et al*[35] fasting G/I ratio was compared to insulin sensitivity measured by the FSIVGTT. It was found that fasting G/I ratio is a highly sensitive and specific measurement of insulin sensitivity.

Insulinogenic index

The insulinogenic index (IGI) is a frequently used index of β-cell function. It is an index of insulin secretion derived from OGTT[49].

IGI = δinsulin (0-30 min)/δglucose (0-30 min)

Insulin is measured in microunits per millilitre, whereas glucose is measured in milligrams per decilitre[49].

The insulinogenic index helps to estimate the level of insulin secretion with a more physiological route of glucose administration.

While it has not been extensively validated,the insulinogenic index during the first 30 min of the OGTT has commonly been used in epidemiological studies as a surrogate measure of first-phase insulin responses to a glucose challenge.

HOMA

HOMA was first developed in 1985 by Matthews *et al*[36]. It is a method used to quantify insulin resistance and beta-cell function from basal (fasting) glucose and insulin (or C-peptide) concentrations. HOMA is a model of the relationship of glucose and insulin dynamics that predicts fasting steady-state glucose and insulin concentrations for a wide range of possible combinations of insulin resistance and β-cell function. Insulin levels depend on the pancreatic β-cell effect to glucose concentrations while, glucose concentrations are regulated by insulin-mediated glucose production via the liver. Thus, deficient β-cell function will echo a diminished response of β-cell to glucose-stimulated insulin secretion[35,50,51]. Similarly, insulin resistance is reflected by the diminished suppressive effect of insulin on hepatic glucose production. The HOMA model has proved to be a robust clinical and epidemiological tool for the assessment of insulin resistance.

HOMA describes this glucose-insulin homeostasis by means of a set of simple, mathematically-derived nonlinear equations. The approximating equation for insulin resistance has been simplified, and uses a fasting blood sample. It is derived from the use of the insulin-glucose product, divided by a constant. The product of FPG × FPI is an index of hepatic insulin resistance.

HOMA-IR = (glucose × insulin)/22.5: Insulin concentration is reported in μU/L and glucose in mmol/L.

The constant of 22.5 is a normalizing factor; i.e, the product of normal fasting plasma insulin of 5 μU/mL, and the normal fasting plasma glucose of 4.5 mmol/L typical of a "normal" healthy individual = 22.5. Whereas the β-cell function is also calculated by another equation using fasting insulin and glucose values.

HOMA1 - %B = (20 × FPI)/(FPG - 3.5): On the other hand, HOMA β-cell is another calculated variable indicating the insulin activity. It is a marker of basal insulin secretion of pancreatic β-cells[52].

HOMA β cell = 20 × fasting plasma insulin (μU/mL)/FPG (mmol)-3: Estimation with the help of HOMA model parallels equally with that of the euglycemic clamp method ($r = 0.88$)[51].

HOMA-IR has been observed to have a linear correlation with the glucose clamp and minimal model estimates of insulin sensitivity/resistance in various studies of distinct populations[51,53]. Derived from a mathematical assessment of the interaction between β-cell function and IR, the HOMA model is used to compute steady-state insulin and glucose concentrations. C-peptide, a measure of insulin secretion (not insulin action), can be used in HOMA modelling of both β-cell function and IR.

QUICKI

QUICKI is an empirically-derived mathematical transformation of fasting blood glucose and plasma insulin concentrations that provides a consistent and precise index of insulin sensitivity with better positive predictive power[41,54-56]. It is simply a variation of HOMA equations, as it transforms the data by taking both the logarithm and the reciprocal of the glucose-insulin product, thus slightly skewing the distribution of fasting insulin values.

QUICKI has been seen to have a significantly better linear correlation with glucose clamp determinations of insulin sensitivity than minimal-model estimates, especially in obese and diabetic subjects[54]. It employs the use of fasting values of insulin and glucose as in HOMA calculations. QUICKI[37] is virtually identical to the simple equation form of the HOMA model in all aspects, except that a log transform of the insulin glucose product is employed to calculate QUICKI.

QUICKI = 1/[log (Insulin μU/mL) + log (Glucose mg/dL)][37].

QUICKI should not be considered, as a new model rather simply logs HOMA-IR, which explains the near-perfect correlation with HOMA. It has similar drawbacks to the use of the HOMA equations, compared with the computer model. Given the similarities between QUICKI and HOMA, the two methods compare well.

In conditions like diabetes, glucose intolerance, and hyperlipidemia associated with insulin resistance, or with various combinations of these metabolic disorders, QUICKI index values have been observed to be lower when compared to those of healthy volunteers. Adult patients with a QUICKI index below 0.357 (which is at the lower limit of 95% confidence limits in healthy people) tend to have a higher risk or frequently present with typical manifestations of metabolic syndrome[57]. Each laboratory should establish its own normal QUICKI range, since variations in insulin determinations of different laboratories is unavoidable.

Minimal model analysis of the frequently sampled intravenous glucose tolerance test

The minimal model is a method to obtain an indirect measurement of metabolic insulin sensitivity/resistance was developed by Bergman *et al*[58] in 1979. Glucose and insulin values obtained during a FSIVGTT are used in this method.

The data collected by this method, which involves multiple blood sampling, is subjected to minimal model analysis, using the computer program MINMOD to generate an index of insulin sensitivity (IS). After an overnight fast, glucose is infused intravenously over 2 min, starting at time 0. Presently, a modified FSIVGTT is used where exogenous insulin is also infused after the intravenous glucose bolus[59-61] followed by the extraction of blood samples for the estimation of plasma glucose and insulin measurements at -10, -1, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 20, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 80, 90, 100, 120, 160, and 180 min.

In contrast to the glucose clamp and IST, which depend on steady-state conditions, the minimal model approach employs the use of dynamic data. Minimal model analysis of the modified FSIVGTT being less demanding in terms of labour, as there are no intravenous infusions and not requirement for steady-state conditions, it is generally found to be easier than the glucose clamp method. The minimal model method[62] is a simple method, but the complexity of the sampling procedure, the sophisticated data analysis, and the correspondingly higher cost make it unsuitable for clinical settings.

Glucose insulin (GI) product

Application of the product of the plasma glucose and insulin concentrations during the OGTT has also been supported by few researchers as an index of whole-body insulin sensitivity[63,64]. IR can be envisaged by increased plasma insulin in spite of normal or increased plasma glucose concentrations. The product of the plasma glucose and insulin concentrations provides the better index of insulin sensitivity. Furthermore, the higher the plasma glucose level, along with a higher plasma insulin response, the more severe is the state of insulin resistance. The lower the GI product, the more responsive are the tissues of the body to insulin. Nonetheless, Matsuda and Defronzo found that this measure correlated well with rate of insulin-mediated glucose disposal during the euglycemic insulin clamp[50].

Fasting insulin resistance index

The fasting insulin resistance index (FIRI) was formulated

Table 2 Various derived surrogate markers of insulin resistance

S No	Method	Measurement	Comments
1	Matsuda index	10 000/√ (fasting G × fasting I) (mean G × mean I)	Represents both hepatic and peripheral tissue sensitivity to insulin.
2	Gutt index	75 000 + (G_0 - G_{120}) (mg/dL) × 0.19 × BW/120 × $Gmean_{(0, 120)}$ (mmol/L) × Log [$Imean_{(0, 120)}$] (mU/L)	Good to predict onset of type 2 diabetes
3	Stumvoll index	0.156 - 0.0000459 × I_{120} (pmol/L) – 0.000321 × I_0 (pmol/L) – 0.00541 × G_{120} (mmol/L)	Utilizes demographic data like age, sex and BMI along with plasma glucose and insulin to predict insulin sensitivity
4	Avignon index	Sib = 10^8/[I_0 (mU/L) × G_0 (mmol/L) × VD) Si2h = 10^8/(I_{120} (mU/L) × G_{120} (mmol/L) × VD]	Determines glucose tolerance and insulin sensitivity in single test
5	Oral glucose insulin sensitivity index	G and I concentrations from a 75 g OGTT at 0, 2, and 3 h (3 h OGTT) or at 0, 1.5, and 2 h (2 h OGTT). The formula includes six constants	
6	Log (HOMA-IR)	Evaluates insulin resistance in insulin-resistant states like glucose intolerance and mild to moderate diabetes	

Sib: Derived from fasting plasma insulin and glucose; Si2h: Derived from fasting plasma insulin and glucose ant 2 h of OGTT; OGTT: Oral glucose tolerance test.

by Duncan *et al*[37] in search of a distinct marker, as the use or ratio of glucose and insulin might not be reliable for the estimation of IR. Increased insulin secretion to restore a normal level of plasma glucose leads to persistent elevation of insulin and probably of glucose also.

FIRI is calculated as FIRI = (fasting glucose × fasting insulin)/25.

Derived surrogate markers

Clinical investigators have been in search of more practical indices that measure insulin sensitivity comparable to that of the euglycemic hyperinsulinemic clamp. Such indices of whole-body insulin sensitivity derived from plasma glucose and insulin concentrations during OGTT reflect both muscle and liver insulin sensitivity (see Table 2).

Matsuda index

Several methods have been described that derive an index of insulin sensitivity from the OGTT. In these methods, the ratio of plasma glucose to insulin concentration during the OGTT is used. A novel assessment of insulin sensitivity that is simple to calculate and provides a reasonable approximation of whole-body insulin sensitivity from the OGTT was developed by Matsuda and Defronzo, and is referred to as the Matsuda index[50]. Here the OGTT index of insulin sensitivity [ISI (composite)] was calculated using both the data of the entire 3 h OGTT and the first 2 h of the test.

The composite whole-body insulin sensitivity index (WBISI), developed by Matsuda and DeFronzo is based on insulin values given in microunits per millilitre (μU/mL) and those of glucose, in milligrams per decilitre (mg/dL) obtained from the OGTT and the corresponding fasting values[50].

WBISI= 10 000/√ (fasting glucose × fasting insulin) (mean glucose × mean insulin)

This index represents a composite of both hepatic and peripheral tissue sensitivity to insulin.

Gutt index

Gutt *et al*[65] also explored the use of OGTT values in order to try and develop an easy measure of insulin sensitivity. A formula for an insulin sensitivity index, ISI (0, 120), that used the fasting (0 min) and 120 min post-oral glucose (OGTT) insulin(I) and glucose(G) concentrations along with body weight (BW) was devised.

$ISI_{(0, 120)}$ = 75 000 + (G_0 - G_{120}) × 0.19 × BW/120 × $Gmean_{(0, 120)}$ × Log [$Imean_{(0, 120)}$] Insulin concentration is expressed in mU/L and glucose concentration is expressed as mg/dL in the numerator and mmol/L in the denominator. It was shown to correlate well with the insulin sensitivity index obtained from the euglycemic hyperinsulinemic clamp.

Stumvoll index

It is now possible to calculate insulin sensitivity and insulin release from simple demographic parameters and values obtained during an OGTT with practical precision.

Stumvoll *et al*[66] proposed use of demographic data like age, sex and basal metabolic rate (BMI) in addition to plasma glucose (mmol/L) and insulin (pmol/L) responses during the OGTT to predict insulin sensitivity and beta cell function.

$ISI_{Stumvoll}$ = 0.156 - 0.0000459 × I_{120} - 0.000321 × I_0 - 0.00541 × G_{120}

$ISI_{Stumvoll}$ = 0.222 - 0.00333 × BMI - 0.0000779 × I_{120} - 0.000422 × Age

The metabolic clearance rate of glucose and ISI calculated by this method included BMI, insulin (120 min), and glucose (90 min).

These parameters correlated better with the measured parameters than the homeostasis model assessment for secretion and resistance[66].

Avignon index

Avignon *et al*[67] tried to compare IS indicesindices which were derived from plasma insulin (I) (mU/L), glucose (G) (mmol/L) and apparent glucose distribution volume in the basal state (Sib), and at the end of second hour OGTT (Si2h). Another insulin sensitivity index (SiM) was calculated by averaging Sib and Si2h.

Table 3 Imminent markers of insulin resistance

S No	Marker
1	Insulin growth factor binding protein-1 (IGFBP-1)
2	sCD36 (solubleCD36)
3	C-reactive protein (CRP)
4	Ferritin
5	Adiponectin
6	Tumour necrosis factor (TNF alpha)
7	Resistin
8	C3 complement
9	Glycosylated hemoglobin (Hb)A1c
10	Protein kinase C (PKC) in microangiopathy
11	Sex hormone-binding globulin (SHBG) in hyperandrogenic syndrome

SiM = [(0.137 × Sib) + Si2h]/2, where Sib = $10^8/(I_0 \times G_0 \times VD)$ and Si2h = $10^8/(I_{120} \times G_{120} \times VD)$ (VD is an estimate of the apparent glucose distribution volume).

It was observed that the results obtained by computation of sensitivity indicesindices from G and I concentrations in the basal state and during a conventional 2 h OGTT were useful for blending both a determination of glucose tolerance and an estimate of insulin sensitivity in a single and simple test.

Oral glucose insulin sensitivity index

Another group of researchers developed an index of insulin sensitivity which was calculated using a model-derived principle from the OGTT glucose and insulin concentration. This index was found to be equivalent to glucose clearance calculated during a clamp[68].

The oral glucose insulin sensitivity index requires glucose and insulin concentrations from a 75 g OGTT at 0, 2, and 3 h (3 h OGTT) or at 0, 1.5, and 2 h (2 h OGTT). The formula includes six constants optimized to match the clamp results. This is validated against the clamp method in subjects with IGT and type 2 diabetes.

Log (HOMA-IR)

Log (HOMA-IR) is useful for the assessment of insulin resistance in insulin-resistant conditions like glucose intolerance and mild to moderate diabetes. In research studies where assessing insulin sensitivity/resistance is of secondary interest, it may be appropriate to use log (HOMA-IR) instead of the direct use of HOMA.

In the case of relentlessly deranged/β-cell function, HOMA-IR may not give an apposite method to evaluate IR. The coefficient of variation for HOMA-IR varies greatly, depending upon the number of fasting samples obtained and the type of insulin assay used[50,51,69,70]. Log (HOMA-IR) transforms the skewed distribution of fasting insulin values to determine a much stronger linear correlation with glucose clamp estimates of insulin sensitivity when extensive ranges of insulin sensitivity/resistance are being studied[37].

Log (HOMA) is being applied broadly in large epidemiological studies, and in clinical research studies[51,52,71].

Imminent markers

With the passing of time and ongoing intensified research, many newer particles are gaining attention as surrogate markers in assessment of IR. In recent times, inflammatory markers have gained popularity in terms of assessment of insulin resistance (Table 3).

Insulin growth factor binding protein-1

Current research has recommended insulin growth factor binding protein-1 (IGFBP-1) as a new potential plasma marker to assess insulin resistance[72]. IGFBP-1 has been found to have a good correlation with FSIVGTT assessment of insulin sensitivity, mainly in children younger than 10 years[72]. However, more studies are required to authenticate the usefulness of this marker. IGFBP-1 levels decline with obesity and IR. Although elevated fasting insulin is less sensitive but more specific, it has been suggested that in young subjects, IGFBP-1 might act as a convenient and susceptible marker of IR. It is an emerging marker which may be useful in this context.

SolubleCD36

Macrophage CD36 is a key proatherogenic molecule that scavenges oxidized low-density lipoprotein, leading to foam cell formation. Hyperglycemia and altered macrophage insulin signaling in insulin resistance leads to increased expression of CD36[73]. SolubleCD36 has been reported to be distinctly elevated in patients with type 2 diabetes and insulin resistance[73].

It is postulated that it might represent a potential marker of IR and its complications.

C-reactive protein

C-reactive protein (CRP) is one of the best studied markers for systemic subclinical inflammation, and may have prognostic value in predicting the future risk of cardiovascular events[74]. In cross-sectional studies, highly sensitive - CRP has been found to correlate with increased triglyceride, decreased HDL, increased blood pressure and increased fasting plasma glucose concentrations, suggesting its association with increased prevalence metabolic syndrome associated with IR[75,76]. Few studies have established the association of CRP with IR independent of obesity[77].

In a recent study, CRP was found to significantly associate with several surrogate measures of IR like fasting insulin, the Raynaud index, the quantitative insulin sensitivity check index, and the McAuley index, HOMA, QUICKI, the Insulin: glucose ratio and the Avignon index in non-diabetics[78]. Because of the simplicity of measurement, stability, and improved high-sensitivity method, CRP may be useful as a clinical measure for identifying individuals at risk for IR[79].

Ferritin

Ferritin is the major intracellular iron storage protein. Recently it has been suggested that when markers of the iron metabolism are elevated, the incidence of the metabolic syndrome is increased[80]. Ferritin has been associated with

both hyperinsulinemia and hypertriglyceridemia. Metabolic disorders are common among patients with high ferritin without genetic hemochromatosis, than among patients with genetic hemochromatosis. Iron deposition in various tissues affects insulin sensitivity and function, thereby leading to insulin resistance and inflammation.

A few studies have demonstrated a link between markers of insulin resistance (HOMA-IR, fasting insulin) and ferritin[81]. Fumeron *et al*[82] also found that plasma ferritin concentrations positively correlate with fasting insulin and fasting glucose.

Adiponectin

Adiponectin is a multifunctional protein that exerts pleiotropic insulin-sensitizing effects and hence is considered as a key molecule in the pathogenesis of metabolic syndrome[83,84]. It lowers hepatic glucose production[85] and increases glucose uptake and fatty acid oxidation in skeletal muscle[86]. Adiponectin levels are decreased in obesity and are inversely correlated to insulin-resistant states and high-sensitivity CRP levels[87].

Deranged levels of adiponectin have been found to be related to insulin resistance. Adiponectin appears to have a stronger negative correlation with HOMA in individuals without the metabolic syndrome as compared to those with metabolic syndrome[88].

Several prospective studies have confirmed that hypoadiponectinemia was associated with an increase in insulin resistance[89] and an elevated risk of developing diabetes[90,91].

Tumour necrosis factor alpha

Several studies have been conducted to explore the role and use of tumour necrosis factor (TNF) to aid in assessing the IR. TNF has been proven to have a relation to insulin resistance measured by HOMA-IR[92] or insulin clamp[93,94] and to metabolic syndrome status[95].

Resistin

The association between resistin and insulin resistance in humans has not been fully established. Many studies have been unsuccessful in recognizing an association between resistin and measures of insulin resistance[96,97]. On the other hand, a few studies have been conducted which have indeed discovered a significant relationship between IR (HOMA-IR) and resistin[88,98-100].

C3 complement

The main activation fragment of C3, C3a desArg (acylation stimulating protein) favours glucose transmembrane transport and the synthesis of triglycerides in adipocytes. This suggests that it has insulin-like properties[101]. C3 is strongly linked with insulin resistance (as defined according to the homeostasis model assessment (HOMA), independent of the components of the metabolic syndrome[102]. The strong association of C3 with insulin action and fasting insulin has been reported in young adult Pima Indians[103].

Glycosylated hemoglobin

Glycosylated hemoglobin (HbA1c) has been used to review long-term glycemic control in diabetics. However, its role and clinical worth in patients suffering from IR or metabolic syndrome in nondiabetic subjects is dubious. HbA1c has been proposed as a measure of surrogate assessment of metabolic syndrome, thereby estimating IR because of various factors. HbA1c reflects long-term glycemic control in diabetic patients and is a significant predictor of long-term complications of diabetes[104,105]. Though HbA1c cannot be considered as a screening or diagnostic tool for diabetes, it has been demonstrated that HbA1c represents both fasting and postprandial glycemic states[106-111].

Upper normal levels of HbA1c in the range of 5.7%-6.4% have been found to echo some components of insulin resistance syndrome or metabolic syndrome[112]. A study conducted in the nondiabetic, obese, first-degree relatives of African-Americans who were genetically predisposed to type 2 diabetes[112] showed significantly high HOMA IR, reduced insulin sensitivity and reduced glucose effectiveness in the nondiabetic study group. Insulin sensitivity and glucose effectiveness were calculated using Bergman's Minmod software program[113,114].

It has been postulated that HbA1c can be considered predictive of insulin resistance.

Protein kinase C in microangiopathy

It has been speculated that activation of the protein kinase C b isoform (PKCb) which is mediated by hyperglycemia acts as a potential surrogate marker for microangiopathic diseases, and diabetic retinopathy in particular[115]. A study conducted on diabetic patients correlated PKC activation with diabetic retinopathy. It was suggested that PKC activation in mononuclear cells may serve as a surrogate marker for diabetic microangiopathy[115].

Sex hormone-binding globulin in hyperandrogenic syndrome

Sex hormone-binding globulin (SHBG) may serve as a predictive marker of IR in obese women suffering from hyperandrogenic syndrome. In a study conducted by Kajaia *et al*[116], IR was established by means of the Matsuda ISI in hyperandrogenic women, who were discovered to have significantly lower SHBG and HDL levels. SHBG may be regarded as an extrapolative marker in these types of cases.

CONCLUSION

To summarize, this article is an attempt to scrutinize a variety of methods currently available for estimating insulin sensitivity/resistance. Assessment of insulin resistance is increasingly being exploited in clinical situations, and this calls for the existence of relatively simple markers. The application of surrogate markers is a useful tool with which to gauge IR. These vary from intricate, time-consuming and invasive procedures, to simple tests

involving a single fasting blood sample. The glucose clamp method has been the reference standard for direct measurement of insulin sensitivity. With regard to simple markers, HOMA and QUICKI are among the best and most extensively validated surrogates that can give a more physiological estimate of glucose homeostasis. Other derived indirect indices have been recognised that correlate well with those derived from clamp studies. It is important to understand the concepts and relative merits and limitations underlying each method in order to correctly interpret the data for measuring insulin sensitivity. Several novel markers like the insulin growth factor binding protein-1, hs-CRP, adiponectin, ferritin, HbA1c, C3 complement, TNF alpha and sCD36 are now surfacing as surrogate markers of IR.

The use of surrogate markers to assess insulin resistance might thus help to use medical resources to fullest, while minimizing costs and inconvenient side effects.

REFERENCES

1 **DeFronzo RA**, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM. A balanced overview. *Diabetes Care* 1992; **15**: 318-368
2 **Berson SA**, Yalow RS. In: Ellenberg M, Rifkin H, editors. Diabetes Mellitus: Theory and Practice, New York: McGraw-Hill,1970; 388-423
3 **Jensen MD**, Caruso M, Heiling V, Miles JM. Insulin regulation of lipolysis in nondiabetic and IDDM subjects. *Diabetes* 1989; **38**: 1595-1601
4 **Eckel RH**. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. *N Engl J Med* 1989; **320**: 1060-1068
5 **Lewis GF**, Steiner G. Acute effects of insulin in the control of VLDL production in humans. Implications for the insulin-resistant state. *Diabetes Care* 1996; **19**: 390-393
6 **Schmidt MI**, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, Azambuja MI, Tracy RP, Heiss G. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *Lancet* 1999; **353**: 1649-1652
7 **Pradhan AD**, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 2001; **286**: 327-334
8 **Fernández-Real JM**, Ricart W. Insulin resistance and chronic cardiovascular inflammatory syndrome. *Endocr Rev* 2003; **24**: 278-301
9 **Reaven GM**. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988; **37**: 1595-1607
10 **Grundy SM**, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004; **109**: 433-438
11 **Olefsky JM**, Saltiel AR. PPAR gamma and the treatment of insulin resistance. *Trends Endocrinol Metab* 2000; **11**: 362-368
12 **Bressler P**, Bailey SR, Matsuda M, DeFronzo RA. Insulin resistance and coronary artery disease. *Diabetologia* 1996; **39**: 1345-1350
13 **Laakso M**, Sarlund H, Salonen R, Suhonen M, Pyorala K, Salonen JT, Karhapaa P. Asymptomatic atherosclerosis and insulin resistance. *Arterioscler Thromb Vasc Biol* 1991; **11**: 1068-1076
14 **Howard G**, O'Leary DH, Zaccaro D, Haffner S, Rewers M, Hamman R, Selby JV, Saad MF, Savage P, Bergman R. Insulin sensitivity and atherosclerosis. The Insulin Resistance Atherosclerosis Study (IRAS) Investigators. *Circulation* 1996; **93**: 1809-1817
15 **Bonora E**, Tessari R, Micciolo R, Zenere M, Targher G, Padovani R, Falezza G, Muggeo M. Intimal-medial thickness of the carotid artery in nondiabetic and NIDDM patients. Relationship with insulin resistance. *Diabetes Care* 1997; **20**: 627-631
16 **Inchiostro S**, Bertoli G, Zanette G, Donadon V. Evidence of higher insulin resistance in NIDDM patients with ischaemic heart disease. *Diabetologia* 1994; **37**: 597-603
17 **Reaven GM**, Chen YD, Jeppesen J, Maheux P, Krauss RM. Insulin resistance and hyperinsulinemia in individuals with small, dense low density lipoprotein particles. *J Clin Invest* 1993; **92**: 141-146
18 **Jeppesen** J, Hollenbeck CB, Zhou MY, Coulston AM, Jones C, Chen YD, Reaven GM. Relation between insulin resistance, hyperinsulinemia, postheparin plasma lipoprotein lipase activity, and postprandial lipemia. *Arterioscler Thromb Vasc Biol* 1995; **15**: 320-324
19 **Facchini F**, Chen YD, Hollenbeck CB, Reaven GM. Relationship between resistance to insulin-mediated glucose uptake, urinary uric acid clearance, and plasma uric acid concentration. *JAMA* 1991; **266**: 3008-3011
20 **Juhan-Vague I**, Thompson SG, Jespersen J. Involvement of the hemostatic system in the insulin resistance syndrome. A study of 1500 patients with angina pectoris. The ECAT Angina Pectoris Study Group. *Arterioscler Thromb* 1993; **13**: 1865-1873
21 **Facchini FS**, Stoohs RA, Reaven GM. Enhanced sympathetic nervous system activity. The linchpin between insulin resistance, hyperinsulinemia, and heart rate. *Am J Hypertens* 1996; **9**: 1013-1017
22 **Barbieri RL**, Ryan KJ. Hyperandrogenism, insulin resistance, and acanthosis nigricans syndrome: a common endocrinopathy with distinct pathophysiologic features. *Am J Obstet Gynecol* 1983; **147**: 90-101
23 **Rao G**. Insulin resistance syndrome. *Am Fam Physician* 2001; **63**: 1559–1563
24 **Dzau VJ**. Markers of malign across the cardiovascular continuum: interpretation and application. *Circulation* 2004; **109**: IV1-IV2
25 **Rosenson RS**. Assessing risk across the spectrum of patients with the metabolic syndrome. *Am J Cardiol* 2005; **96**: 8E-10E
26 **Accili D**. Lilly lecture 2003: the struggle for mastery in insulin action: from triumvirate to republic. *Diabetes* 2004; **53**: 1633-1642
27 **Prodi E**, Obici S. Minireview: the brain as a molecular target for diabetic therapy. *Endocrinology* 2006; **147**: 2664-2669
28 **Kahn SE**, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* 2006; **444**: 840-846
29 **Muniyappa R**, Montagnani M, Koh KK, Quon MJ. Cardiovascular actions of insulin. *Endocr Rev* 2007; **28**: 463-491
30 **Muniyappa R**, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am J Physiol Endocrinol Metab* 2008; **294**: E15-E26
31 **Yeni-Komshian H**, Carantoni M, Abbasi F, Reaven GM. Relationship between several surrogate estimates of insulin resistance and quantification of insulin-mediated glucose disposal in 490 healthy nondiabetic volunteers. *Diabetes Care* 2000; **23**: 171-175
32 **Robbins DC**, Andersen L, Bowsher R, Chance R, Dinesen B, Frank B, Gingerich R, Goldstein D, Widemeyer HM, Haffner S, Hales CN, Jarett L, Polonsky K, Porte D, Skyler J, Webb G, Gallagher K. Report of the American Diabetes Association's Task Force on standardization of the insulin assay. *Diabetes* 1996; **45**: 242-256
33 **Thomas GN**, Critchley JA, Tomlinson B, Anderson PJ, Lee

ZS, Chan JC. Obesity, independent of insulin resistance, is a major determinant of blood pressure in normoglycemic Hong Kong Chinese. *Metabolism* 2000; **49**: 1523-1528
34 **Laakso M**. How good a marker is insulin level for insulin resistance? *Am J Epidemiol* 1993; **137**: 959-965
35 **Legro RS**, Finegood D, Dunaif A. A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. *J Clin Endocrinol Metab* 1998; **83**: 2694-2698
36 **Matthews DR**, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412-419
37 **Duncan MH**, Singh BM, Wise PH, Carter G, Alaghband-Zadeh J. A simple measure of insulin resistance. *Lancet* 1995; **346**: 120-121
38 **Katz A**, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 2000; **85**: 2402-2410
39 **Hanson RL**, Pratley RE, Bogardus C, Narayan KM, Roumain JM, Imperatore G, Fagot-Campagna A, Pettitt DJ, Bennett PH, Knowler WC. Evaluation of simple indices of insulin sensitivity and insulin secretion for use in epidemiologic studies. *Am J Epidemiol* 2000; **151**: 190-198
40 **Mather KJ**, Hunt AE, Steinberg HO, Paradisi G, Hook G, Katz A, Quon MJ, Baron AD. Repeatability characteristics of simple indices of insulin resistance: implications for research applications. *J Clin Endocrinol Metab* 2001; **86**: 5457-5464
41 **DeFronzo RA**, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979; **237**: E214- E223
42 Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2007; **30** Suppl 1: S42- S47
43 **Dalla Man C,** Campioni M, Polonsky KS, Basu R, Rizza RA, Toffolo G, Cobelli C. Two-hour seven-sample oral glucose tolerance test and meal protocol: minimal model assessment of beta-cell responsivity and insulin sensitivity in nondiabetic individuals. *Diabetes* 2005; **54**: 3265-3273
44 **Hsieh CH**, Kuo SW, Hung YJ, Shen DC, Ho CT, Lian WC, Lee CH, Fan SC, Pei D. Metabolic characteristics in individuals with impaired glucose homeostasis. *Int J Clin Pract* 2005; **59**: 639-644
45 Consensus Development Conference on Insulin Resistance. 5-6 November 1997. American Diabetes Association. *Diabetes Care* 1998; **21**: 310-314
46 **Silfen ME**, Manibo AM, McMahon DJ, Levine LS, Murphy AR, Oberfield SE. Comparison of simple measures of insulin sensitivity in young girls with premature adrenarche: the fasting glucose to insulin ratio may be a simple and useful measure. *J Clin Endocrinol Metab* 2001; **86**: 2863-2868
47 **Vuguin P**, Saenger P, Dimartino-Nardi J. Fasting glucose insulin ratio: a useful measure of insulin resistance in girls with premature adrenarche. *J Clin Endocrinol Metab* 2001; **86**: 4618-4621
48 **Quon MJ**. Limitations of the fasting glucose to insulin ratio as an index of insulin sensitivity. *J Clin Endocrinol Metab* 2001; **86**: 4615-4617
49 **Goedecke JH**, Dave JA, Faulenbach MV, Utzschneider KM, Lambert EV, West S, Collins M, Olsson T, Walker BR, Seckl JR, Kahn SE, Levitt NS. Insulin response in relation to insulin sensitivity: an appropriate beta-cell response in black South African women. *Diabetes Care* 2009; **32**: 860-865
50 **Matsuda M**, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999; **22**: 1462-1470
51 **Wallace TM**, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004; **27**: 1487-1495
52 **Haffner SM**, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. *Diabetes Care* 1997; **20**: 1087-1092
53 **Radziuk J**. Insulin sensitivity and its measurement: structural commonalities among the methods. *J Clin Endocrinol Metab* 2000; **85**: 4426-4433
54 **Chen H**, Sullivan G, Yue LQ, Katz A, Quon MJ. QUICKI is a useful index of insulin sensitivity in subjects with hypertension. *Am J Physiol Endocrinol Metab* 2003; **284**: E804-E812
55 **Chen H**, Sullivan G, Quon MJ. Assessing the predictive accuracy of QUICKI as a surrogate index for insulin sensitivity using a calibration model. *Diabetes* 2005; **54**: 1914-1925
56 **Hanley AJ**, Williams K, Gonzalez C, D'Agostino RB Jr, Wagenknecht LE, Stern MP, Haffner SM. Prediction of type 2 diabetes using simple measures of insulin resistance: combined results from the San Antonio Heart Study, the Mexico City Diabetes Study, and the Insulin Resistance Atherosclerosis Study. *Diabetes* 2003; **52**: 463-469
57 **Hrebícek J**, Janout V, Malincíková J, Horáková D, Cízek L. Detection of insulin resistance by simple quantitative insulin sensitivity check index QUICKI for epidemiological assessment and prevention. *J Clin Endocrinol Metab* 2002; **87**: 144-147
58 **Bergman RN**, Ider YZ, Bowden CR, Cobelli C. Quantitative estimation of insulin sensitivity. *Am J Physiol* 1979; **236**: E667-E677
59 **Finegood DT**, Hramiak IM, Dupre J. A modified protocol for estimation of insulin sensitivity with the minimal model of glucose kinetics in patients with insulin-dependent diabetes. *J Clin Endocrinol Metab* 1990; **70**: 1538-1549
60 **Quon MJ**, Cochran C, Taylor SI, Eastman RC. Direct comparison of standard and insulin modified protocols for minimal model estimation of insulin sensitivity in normal subjects. *Diabetes Res* 1994; **25**: 139-149
61 **Saad MF**, Steil GM, Kades WW, Ayad MF, Elsewafy WA, Boyadjian R, Jinagouda SD, Bergman RN. Differences between the tolbutamide-boosted and the insulin-modified minimal model protocols. *Diabetes* 1997; **46**: 1167-1171
62 **Bergman RN**, Prager R, Volund A, Olefsky JM. Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. *J Clin Invest* 1987; **79**: 790-800
63 **Levine R**, Haft DE. Carbohydrate homeostasis. *N Engl J Med* 1970; **283**: 237-246
64 **Myllynen P**, Koivisto VA, Nikkilä EA. Glucose intolerance and insulin resistance accompany immobilization. *Acta Med Scand* 1987; **222**: 75-81
65 **Gutt M**, Davis CL, Spitzer SB, Llabre MM, Kumar M, Czarnecki EM, Schneiderman N, Skyler JS, Marks JB. Validation of the insulin sensitivity index (ISI(0,120)): comparison with other measures. *Diabetes Res Clin Pract* 2000; **47**: 177-184
66 **Stumvoll M**, Mitrakou A, Pimenta W, Jenssen T, Yki-Järvinen H, Van Haeften T, Renn W, Gerich J. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. *Diabetes Care* 2000; **23**: 295-301
67 **Avignon A**, Boegner C, Mariano-Goulart D, Colette C, Monnier L. Assessment of insulin sensitivity from plasma insulin and glucose in the fasting or post oral glucose-load state. *Int J Obes Relat Metab Disord* 1999; **23**: 512-517
68 **Mari A**, Pacini G, Murphy E, Ludvik B, Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes Care* 2001; **24**: 539-548
69 **Bonora E**, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T, Muggeo M. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care* 2000; **23**: 57-63
70 **Emoto M**, Nishizawa Y, Maekawa K, Hiura Y, Kanda H, Kawagishi T, Shoji T, Okuno Y, Morii H. Homeostasis model

assessment as a clinical index of insulin resistance in type 2 diabetic patients treated with sulfonylureas. *Diabetes Care* 1999; **22**: 818-822
71 **Dabelea D**, Pettitt DJ, Hanson RL, Imperatore G, Bennett PH, Knowler WC. Birth weight, type 2 diabetes, and insulin resistance in Pima Indian children and young adults. *Diabetes Care* 1999; **22**: 944-950
72 **Motaghedi R**, Gujral S, Sinha S, Sison C, Ten S, Maclaren NK. Insulin-like growth factor binding protein-1 to screen for insulin resistance in children. *Diabetes Technol Ther* 2007; **9**: 43-51
73 **Handberg A**, Levin K, Højlund K, Beck-Nielsen H. Identification of the oxidized low-density lipoprotein scavenger receptor CD36 in plasma: a novel marker of insulin resistance. *Circulation* 2006; **114**: 1169-1176
74 **Haverkate F**, Thompson SG, Pyke SD, Gallimore JR, Pepys MB. Production of C-reactive protein and risk of coronary events in stable and unstable angina. European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. *Lancet* 1997; **349**: 462-466
75 **Mendall MA**, Patel P, Asante M, Ballam L, Morris J, Strachan DP, Camm AJ, Northfield TC. Relation of serum cytokine concentrations to cardiovascular risk factors and coronary heart disease. *Heart* 1997; **78**: 273-277
76 **Pasceri V**, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation* 2000; **102**: 2165-2168
77 **Taniguchi A**, Nagasaka S, Fukushima M, Sakai M, Okumura T, Yoshii S, Watanabe T, Ogura M, Yamadori N, Nin K, Kuroe A, Yamada Y, Seino Y, Nakai Y. C-reactive protein and insulin resistance in non-obese Japanese type 2 diabetic patients. *Metabolism* 2002; **51**: 1578-1581
78 **Meng YX**, Ford ES, Li C, Quarshie A, Al-Mahmoud AM, Giles W, Gibbons GH, Strayhorn G. Association of C-reactive protein with surrogate measures of insulin resistance among nondiabetic US from National Health and Nutrition Examination Survey 1999-2002. *Clin Chem* 2007; **53**: 2152-2159
79 **Ridker PM**, Wilson PW, Grundy SM. Should C-reactive protein be added to metabolic syndrome and to assessment of global cardiovascular risk? *Circulation* 2004; **109**: 2818-2825
80 **Vari IS**, Balkau B, Kettaneh A, André P, Tichet J, Fumeron F, Caces E, Marre M, Grandchamp B, Ducimetière P. Ferritin and transferrin are associated with metabolic syndrome abnormalities and their change over time in a general population: Data from an Epidemiological Study on the Insulin Resistance Syndrome (DESIR). *Diabetes Care* 2007; **30**: 1795-1801
81 **Jehn M**, Clark JM, Guallar E. Serum ferritin and risk of the metabolic syndrome in U.S. adults. *Diabetes Care* 2004; **27**: 2422-2428
82 **Fumeron F**, Péan F, Driss F, Balkau B, Tichet J, Marre M, Grandchamp B. Ferritin and transferrin are both predictive of the onset of hyperglycemia in men and women over 3 years: the data from an epidemiological study on the Insulin Resistance Syndrome (DESIR) study. *Diabetes Care* 2006; **29**: 2090-2094
83 **Matsuzawa Y**, Funahashi T, Kihara S, Shimomura I. Adiponectin and metabolic syndrome. *Arterioscler Thromb Vasc Biol* 2004; **24**: 29-33
84 **Ryo M**, Nakamura T, Kihara S, Kumada M, Shibazaki S, Takahashi M, Nagai M, Matsuzawa Y, Funahashi T. Adiponectin as a biomarker of the metabolic syndrome. *Circ J* 2004; **68**: 975-981
85 **Berg AH**, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 2001; **7**: 947-953
86 **Yamauchi T**, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; **8**: 1288-1295
87 **Higashiura K**, Ura N, Ohata J, Togashi N, Takagi S, Saitoh S, Murakami H, Takagawa Y, Shimamoto K. Correlations of adiponectin level with insulin resistance and atherosclerosis in Japanese male populations. *Clin Endocrinol* (Oxf) 2004; **61**: 753-759
88 **Hivert MF**, Sullivan LM, Fox CS, Nathan DM, D'Agostino RB Sr, Wilson PW, Meigs JB. Associations of adiponectin, resistin, and tumor necrosis factor-alpha with insulin resistance. *J Clin Endocrinol Metab* 2008; **93**: 3165-3172
89 **Yamamoto Y**, Hirose H, Saito I, Nishikai K, Saruta T. Adiponectin, an adipocyte-derived protein, predicts future insulin resistance: two-year follow-up study in Japanese population. *J Clin Endocrinol Metab* 2004; **89**: 87-90
90 **Duncan BB**, Schmidt MI, Pankow JS, Bang H, Couper D, Ballantyne CM, Hoogeveen RC, Heiss G. Adiponectin and the development of type 2 diabetes: the atherosclerosis risk in communities study. *Diabetes* 2004; **53**: 2473-2478
91 **Snijder MB**, Heine RJ, Seidell JC, Bouter LM, Stehouwer CD, Nijpels G, Funahashi T, Matsuzawa Y, Shimomura I, Dekker JM. Associations of adiponectin levels with incident impaired glucose metabolism and type 2 diabetes in older men and women: the hoorn study. *Diabetes Care* 2006; **29**: 2498-2503
92 **Zinman B**, Hanley AJ, Harris SB, Kwan J, Fantus IG. Circulating tumor necrosis factor-alpha concentrations in a native Canadian population with high rates of type 2 diabetes mellitus. *J Clin Endocrinol Metab* 1999; **84**: 272-278
93 **Behre CJ**, Fagerberg B, Hultén LM, Hulthe J. The reciprocal association of adipocytokines with insulin resistance and C-reactive protein in clinically healthy men. *Metabolism* 2005; **54**: 439-444
94 **Miyazaki Y**, Pipek R, Mandarino LJ, DeFronzo RA. Tumor necrosis factor alpha and insulin resistance in obese type 2 diabetic patients. *Int J Obes Relat Metab Disord* 2003; **27**: 88-94
95 **Matsushita K**, Yatsuya H, Tamakoshi K, Wada K, Otsuka R, Takefuji S, Sugiura K, Kondo T, Murohara T, Toyoshima H. Comparison of circulating adiponectin and proinflammatory markers regarding their association with metabolic syndrome in Japanese men. *Arterioscler Thromb Vasc Biol* 2006; **26**: 871-876
96 **Lee JH**, Chan JL, Yiannakouris N, Kontogianni M, Estrada E, Seip R, Orlova C, Mantzoros CS. Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. *J Clin Endocrinol Metab* 2003; **88**: 4848-4856
97 **Vozarova de Courten B**, Degawa-Yamauchi M, Considine RV, Tataranni PA. High serum resistin is associated with an increase in adiposity but not a worsening of insulin resistance in Pima Indians. *Diabetes* 2004; **53**: 1279-1284
98 **Ohmori R**, Momiyama Y, Kato R, Taniguchi H, Ogura M, Ayaori M, Nakamura H, Ohsuzu F. Associations between serum resistin levels and insulin resistance, inflammation, and coronary artery disease. *J Am Coll Cardiol* 2005; **46**: 379-380
99 **Silha JV**, Krsek M, Skrha JV, Sucharda P, Nyomba BL, Murphy LJ. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. *Eur J Endocrinol* 2003; **149**: 331-335
100 **Osawa H**, Tabara Y, Kawamoto R, Ohashi J, Ochi M, Onuma H, Nishida W, Yamada K, Nakura J, Kohara K, Miki T, Makino H. Plasma resistin, associated with single nucleotide polymorphism -420, is correlated with insulin resistance, lower HDL cholesterol, and high-sensitivity C-reactive protein in the Japanese general population. *Diabetes Care* 2007; **30**: 1501-1506
101 **Germinario R**, Sniderman AD, Manuel S, Lefebvre SP, Baldo A, Cianflone K. Coordinate regulation of triacylglycerol

synthesis and glucose transport by acylation-stimulating protein. *Metabolism* 1993; **42**: 574-580
102 **Muscari A**, Antonelli S, Bianchi G, Cavrini G, Dapporto S, Ligabue A, Ludovico C, Magalotti D, Poggiopollini G, Zoli M. Serum C3 is a stronger inflammatory marker of insulin resistance than C-reactive protein, leukocyte count, and erythrocyte sedimentation rate: comparison study in an elderly population. *Diabetes Care* 2007; **30**: 2362-2368
103 **Weyer C**, Tataranni PA, Pratley RE. Insulin action and insulinemia are closely related to the fasting complement C3, but not acylation stimulating protein concentration. *Diabetes Care* 2000; **23**: 779-785
104 **Stratton IM**, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ* 2000; **321**: 405-412
105 The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med* 1993; **329**: 977-986
106 **Peters AL**, Davidson MB, Schriger DL, Hasselblad V. A clinical approach for the diagnosis of diabetes mellitus: an analysis using glycosylated hemoglobin levels. Meta-analysis Research Group on the Diagnosis of Diabetes Using Glycated Hemoglobin Levels. *JAMA* 1996; **276**: 1246-1252
107 **Rohlfing CL**, Little RR, Wiedmeyer HM, England JD, Madsen R, Harris MI, Flegal KM, Eberhardt MS, Goldstein DE. Use of GHb (HbA1c) in screening for undiagnosed diabetes in the U.S. population. *Diabetes Care* 2000; **23**: 187-191
108 **Rohlfing CL**, Wiedmeyer HM, Little RR, England JD, Tennill A, Goldstein DE. Defining the relationship between plasma glucose and HbA(1c): analysis of glucose profiles and HbA(1c) in the Diabetes Control and Complications Trial. *Diabetes Care* 2002; **25**: 275-288
109 **Monnier L**, Lapinski H, Colette C. Contributions of fasting and postprandial plasma glucose increments to the overall diurnal hyperglycemia of type 2 diabetic patients: variations with increasing levels of HbA(1c). *Diabetes Care* 2003; **26**: 881-885
110 **Saaddine JB**, Fagot-Campagna A, Rolka D, Narayan KM, Geiss L, Eberhardt M, Flegal KM. Distribution of HbA(1c) levels for children and young adults in the U.S.: Third National Health and Nutrition Examination Survey. *Diabetes Care* 2002; **25**: 1326-1330
111 **Yates AP**, Laing I. Age-related increase in haemoglobin A1c and fasting plasma glucose is accompanied by a decrease in beta cell function without change in insulin sensitivity: evidence from a cross-sectional study of hospital personnel. *Diabet Med* 2002; **19**: 254-258
112 **Osei K**, Rhinesmith S, Gaillard T, Schuster D. Is glycosylated hemoglobin A1c a surrogate for metabolic syndrome in nondiabetic, first-degree relatives of African-American patients with type 2 diabetes? *J Clin Endocrinol Metab* 2003; **88**: 4596-4601
113 **Bergman RN**. Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. *Diabetes* 1989; **38**: 1512-1527
114 **Finegood DT**, Hramiak IM, Dupre J. A modified protocol for estimation of insulin sensitivity with the minimal model of glucose kinetics in patients with insulin-dependent diabetes. *J Clin Endocrinol Metab* 1990; **70**: 1538-1549
115 **Sotiropoulos K**. Protein Kinase C (PKC) Activation in Circulating Mononuclear Cells - Potential Surrogate Marker for Diabetic Retinopathy and Other Microangiopathic Diseases. *Invest Ophthalmol Vis Sci* 2002; **43**: A557
116 **Kajaia N**, Binder H, Dittrich R, Oppelt PG, Flor B, Cupisti S, Beckmann MW, Mueller A. Low sex hormone-binding globulin as a predictive marker for insulin resistance in women with hyperandrogenic syndrome. *Eur J Endocrinol* 2007; **157**: 499-507

S- Editor Zhang HN **L- Editor** Herholdt A **E- Editor** Liu N

World Journal of Diabetes

Online Submissions: http://www.wjgnet.com/1948-9358office
wjd@wjgnet.com
doi:10.4239/wjd.v1.i2.48

World J Diabetes 2010 May 15; 1(2): 48-56
ISSN 1948-9358 (online)

TOPIC HIGHLIGHT

Sarika Arora, MD, Assistant Professor, *Series Editor*

Renal function in diabetic nephropathy

Pradeep Kumar Dabla

Pradeep Kumar Dabla, Department of Biochemistry, Lady Hardinge Medical College, New Delhi 110001, India
Author contributions: Dabla PK contributed solely to this paper.
Correspondence to: Pradeep Kumar Dabla, MD, Postal Paradise Apartment, Flat-428, Block-E, Pocket-3, Sector-18, Rohini, New Delhi 110085, India. pradeep_dabla@yahoo.com
Telephone: +91-98-68524455
Received: December 25, 2009 Revised: April 25, 2010
Accepted: May 2, 2010
Published online: May 15, 2010

Abstract

Diabetic nephropathy is the kidney disease that occurs as a result of diabetes. Cardiovascular and renal complications share common risk factors such as blood pressure, blood lipids, and glycemic control. Thus, chronic kidney disease may predict cardiovascular disease in the general population. The impact of diabetes on renal impairment changes with increasing age. Serum markers of glomerular filtration rate and microalbuminuria identify renal impairment in different segments of the diabetic population, indicating that serum markers as well as microalbuminuria tests should be used in screening for nephropathy in diabetic older people. The American Diabetes Association and the National Institutes of Health recommend Estimated glomerular filtration rate (eGFR) calculated from serum creatinine at least once a year in all people with diabetes for detection of kidney dysfunction. eGFR remains an independent and significant predictor after adjustment for conventional risk factors including age, sex, duration of diabetes, smoking, obesity, blood pressure, and glycemic and lipid control, as well as presence of diabetic retinopathy. Cystatin-C (Cys C) may in future be the preferred marker of diabetic nephropathy due differences in measurements of serum creatinine by various methods. The appropriate reference limit for Cys C in geriatric clinical practice must be defined by further research. Various studies have shown the importance of measurement of albuminuria, eGFR, serum creatinine and hemoglobin level to further enhance the prediction of end stage renal disease.

Key words: Chronic kidney disease; End stage renal disease; Glomerular filtration rate; Estimated glomerular filtration rate; Microalbumin; Cockcroft-Gault formula; Modification of diet; Renal disease; Cystatin-C

Peer reviewer: Richard E Katholi, MD, Prairie Cardiovascular Consultants, Clinical Professor of Pharmacology and Medicine, Southern Illinois University School of Medicine, Springfield, IL 62794-9420, United States

Dabla PK. Renal function in diabetic nephropathy. *World J Diabetes* 2010; 1(2): 48-56 Available from: URL: http://www.wjgnet.com/1948-9358/full/v1/i2/48.htm DOI: http://dx.doi.org/10.4239/wjd.v1.i2.48

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Type 2 diabetes mellitus has quickly become a global health problem due to rapidly increasing population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity. There is, therefore, an urgent need to prevent diabetes and its complications. Diabetes is the major cause of end-stage renal disease (ESRD) both in the U.S. and around the world and has enormous medical, social and economic consequences. Diabetes affects the kidney in stages. At the onset of diabetes, the kidney grows large and the glomerular filtration rate (GFR) becomes disturbed. Most recent basic and clinical research has pointed toward sclerosis and kidney failure. The morbidity and mortality

caused by diabetes mellitus can be reduced by regular screening, early detection, and appropriate treatment of chronic complications. Thus, this discussion will focus on development of kidney damage, the various markers available and approaches to development of future markers to enhance the detection of ESRD at the earliest possible stage.

EPIDEMIOLOGY OF RENAL FAILURE

The global prevalence of diabetes is expected to increase from 4% in 1995 to 5.4% by the year 2025[1]. Currently, the countries with the largest number of diabetic patients are India, China and United States. The acute and chronic complications of diabetes mellitus are major causes of hospital admissions. Asian patients have shown evidence of macro and micro vascular disease at the time of diagnosis of diabetes when compared to Europeans[2].

Diabetes is the most common cause of kidney failure, accounting for nearly 44 percent of new cases[3]. Even when diabetes is controlled, the disease can lead to chronic kidney disease (CKD) and kidney failure. Kidney failure is the final stage of chronic kidney disease. Nearly 24 million people in the United States have diabetes and nearly 180 000 people are living with kidney failure as a result of diabetes[4]. The prevalence of nephropathy in India was less (8.9% in Vellore, 5.5% in Chennai) when compared with the prevalence of 22.3% in Asian Indians in the UK[5]. In chronic renal failure patients the prevalence of diabetic nephropathy was 30.3% followed by chronic interstitial nephritis (23%) and chronic glomerulonephritis (17.7%)[6].

African Americans, American Indians, and Hispanics or Latinos develop diabetes, CKD, and kidney failure at rates higher than Caucasians. Scientists have not been able to explain these higher rates and the interplay of various risk factors.

RISK FACTORS FOR DEVELOPMENT OF DIABETIC COMPLICATIONS

Diabetic nephropathy is the kidney disease that occurs as a result of diabetes. Nephropathy is the leading cause of chronic renal failure worldwide and is responsible for renal failure in about one third of patients who undergo dialysis. It is suggested that patients with common risk factors including greater duration of diabetes, hypertension, poor metabolic control, smoking, obesity and hyperlipidemia are more prone to develop diabetic complications[7]. In a retrospective study done by Klag *et al*[8] it was found that elevations of blood pressure are a strong independent risk factor for end-stage renal disease and that interventions to prevent the disease need to emphasize the prevention and control of both high-normal and high blood pressure.

The "Asian Indian Phenotype" refers to certain unique clinical and biochemical abnormalities in Indians which include increased insulin resistance, higher waist circumference despite lower body mass index, lower adiponectin and higher C-reactive protein levels. This phenotype makes Asians more prone to diabetes and premature complications. Asians show a trend towards higher systolic and diastolic blood pressures, possibly due to more patients with nephropathy, although this is not significantly different. Total cholesterol and LDL cholesterol levels were very similar between Europeans, Americans and Asians, but HDL cholesterol was significantly lower and triglycerides level was significantly higher in Asian patients[9].

James Sowers *et al* reviewed aspects of the association of diabetes with renal disease, emphasizing that CKD and albuminuria are associated with increased rates of cardiovascular disease (CVD) and mortality[10], and should be considered part of the cardiovascular risk factors in persons with diabetes. Lorenzo *et al*[11], found that the development of glomerular filtration rate < 60 mL/min per 1.73 m^2 was associated with increased fasting insulin, triglycerides, free fatty acids, and uric acid and also with antihypertensive treatment, although not with waist circumference, controlling for age, sex, ethnicity, blood pressure, glucose, and C-reactive protein in nondiabetic persons. This points towards the association of CKD with the risk of development of diabetes.

PATHOGENESIS OF KIDNEY DAMAGE

There are various mechanisms of albuminuria which involve abnormalities of the glomerular endothelial barrier[12], causing excessive filtration as well as reduction of renal tubular cell albumin degradation and reabsorption. Glomerular hypertension, inflammation, and oxidative stress worsen albuminuria, with angiotensin-Ⅱ[13] and mechanical stress factors contributing to these processes.

Renal disease in diabetes is found to be associated with abnormalities of vasodilatation and generates reactive oxygen species mediated by endothelial derived nitric oxide (NO), suggesting linkage between vascular and metabolic abnormalities. Angiotensin Ⅱ and aldosterone, interacting with pulse pressure and increased systolic blood pressure, activate NADP oxidase, which acts as mediator of oxidative stress. Angiotensin Ⅱ increases metabolism of NO to peroxynitrite[13], which further impairs endothelial-derived vasodilation.

In another mechanism, decrease in the ability to produce endothelial progenitor cells (EPCs), which can be quantitated with the cellular marker CD 34, leads to increased CVD risk. These cells derived from bone marrow, play a role in replacing damaged endothelium and are reduced in people with decreased endothelium-dependent vasodilation[14]. Both angiotensin Ⅱ and aldosterone inhibit production of EPCs, while angiotensin converting enzyme inhibitors (ACEIs) increase their levels.

Sowers *et al*[15] discussed the relationship between dyslipidemia and CKD, hypothesizing that the mechanism

Table 1 The various tests for chronic tubular dysfunction in diabetic nephropathy

Test name	Use
Blood urea nitrogen (serum or plasma) Method: Spectrophotometry	Initial diagnosis of acute or chronic kidney disease
Creatinine (serum or plasma) Method: Spectrophotometry	Initial diagnosis of acute or chronic kidney disease
Microalbumin (urine) Method: Immunoturbidimetric	May be used as a screening test Useful in diabetic patients to assess baseline renal function Useful in monitoring diabetic nephropathy in insulin-dependent diabetes mellitus
Creatinine based glomerular filtration rate (estimated) Method: Spectrophotometry	Estimate renal function and use as monitoring tool (Test reports serum creatinine reference intervals)
Cystatin-C based glomerular filtration rate (estimated) Method: Nephelometry	May be useful sensitive marker of renal disease; however, test lacks specificity due to reference range inavailability
Retinol-binding protein 4 (RBP4) Method: Non-commercial enzyme-linked immunosorbant assay (ELISA)	May be used as a marker for early diabetic nephropathy. Limited studies are available
Adiponectin Method: Competitive radioimmunoassay	Shown inverse correlation with renal dysfunction in type 2 diabetes
Connective tissue growth factor (CTGF) Method: ELISA	CTGF excretion is correlated inversely with GFRs
Alpha-1-microglobulin (urine) Method: Nephelometry	May indicate renal involvement in diabetic patients
Liver type fatty acid binding protein (L-FABP) Method: ELISA	Expressed in proximal tubular cells and may associated with severity of diabetic nephropathy. Larger conclusive studies are required

of action of statins is an increase in endothelial NO synthase transcription *via* the phosphatidylinositol 3-kinase (PI3K) pathway and statin-induced inhibition of the mobilization of small molecular weight G-proteins. Also, Sowers suggested that the direct renin inhibitor aliskiren has similar benefits in renal disease to those of angiotensin receptor binders.

Charles Heilig *et al*[16] discussed the relationship of renal glucose transporter expression in relation to the development of diabetic nephropathy. Expression of GLUT1, the major mesangial glucose transporter, regulates extracellular matrix production. Mesangial cells overexpressing GLUT1 show increased production of both types Ⅰ and Ⅳ collagen, as well as increased fibronectin and laminin production, leading to a phenotype similar to that of diabetes. In animal models, GLUT1 overexpression in glomeruli creates a nephropathy phenotype resembling that of diabetic renal disease with increased mean glomerular volume, mesangial expansion, and sclerosis.

DEVELOPEMENT OF KIDNEY DISEASE

Diabetic nephropathy takes many years to develop. In some people, the filtering function of the kidneys is actually higher than normal in the first few years of their diabetes.

In people who are developing kidney disease small amounts of the blood protein albumin begin to leak into the urine, a condition called microalbuminuria. The kidney's filtration function usually remains normal during this period. As the disease progresses further, more albumin leaks into the urine, a stage known as macroalbuminuria or proteinuria. As the amount of albumin in the urine increases, the kidney's filtering function usually begins to drop, resulting in the body's retention of various wastes. As kidney damage develops, blood pressure also often rises or hypertension may attenuate the process of renal injury. Early detection of renal damage may help to delay the process.

As these processes are slow, kidney damage rarely occurs in the first 10 years of diabetes, and 15 to 25 years will usually pass before kidney failure occurs. For people who live with diabetes for more than 25 years without any signs of kidney failure, the risk of ever developing it decreases.

BIOMARKERS OF CHRONIC TUBULAR DYSFUNCTION

The early detection of diabetic nephropathy, resulting in timely intervention with particular attention to blood pressure control (thus limiting proteinuria), glycemic control, smoking cessation, and accentuation of cardiovascular risk, can improve long-term outcomes and retard progression to ESRD.

Tubular proteinuria results when glomerular function is normal but the proximal tubules have diminished absorbing capacity. The biomarkers of this process are as Table 1.

Blood urea nitrogen

Blood tests for Blood urea nitrogen (BUN) and creatinine are the simplest way to monitor kidney function. These substances are normal metabolic waste products that are excreted by the kidneys. Urea is a byproduct of protein breakdown. A test can be done to measure the amount of urea nitrogen in the blood. In kidney disease, these substances (as well as numerous others) are not excreted normally, and so they accumulate in the body thus causing an increase in blood levels of urea. The normal level of BUN is 7-20 mg/dL[17].

Creatinine

Serum creatinine is primarily a metabolite of creatine, almost all of which is located in skeletal muscle. The normal level of creatinine is 0.8 to 1.4 mg/dL. Females usually have a lower creatinine (0.6 to 1.2 mg/dL) than males, because they usually have less muscle mass[17].

The amount of creatine per unit of skeletal muscle mass is consistent and the breakdown rate of creatine is also consistent. Thus, plasma creatinine concentration

is very stable and a direct reflection of skeletal muscle mass[18]. Interestingly, Nobuko Harita *et al*[19], hypothesized that, lower serum creatinine is associated with an increased risk of type 2 diabetes, which might reflect a lower volume of skeletal muscle. Skeletal muscle is a major target tissue of insulin and a lower volume of skeletal muscle would mean fewer target sites for insulin which causes increase in insulin resistance. This leads to the development of type 2 diabetes[20]. This may explain in part the pathogenesis of type 2 diabetes associated with lower serum creatinine.

Creatinine based GFR

GFR is the best measure of kidney function since it accounts for age, BMI and sex. GFR measures the rate at which the kidneys' two million glomeruli filter plasma in order to process it and remove waste products from it. If the kidneys are injured by chronic kidney disease, the GFR gradually declines, and the amount of remaining kidney function can be estimated by measuring or calculating the GFR. The normal value for GFR in a normal-sized person is 100-150 mL/min.

Currently the two most common methods for determining GFR are creatinine clearance and estimated GFR (eGFR).

Creatinine clearance: Creatinine clearance requires a 24 h urine collection. A blood sample is drawn at some point during the 24 h period, and creatinine clearance, can then be calculated. However, because a small amount of creatinine is released by the filtering tubes in the kidneys, creatinine clearance is not exactly the same as the GFR. In fact, creatinine clearance usually overestimates the GFR, particularly in patients with advanced kidney failure. Normal clearance values are: Male: 97 to 137 mL/min; Female: 88 to 128 mL/min[21].

There are several factors that may interfere with the accuracy of the test. These include: (1) Incomplete urine collection; (2) Pregnancy; and (3) Vigorous exercise. Creatinine clearance measurements can also be affected by drugs, such as: cimetidine, trimethoprim, and drugs that can damage the kidneys (cephalosporins).

The creatinine clearance test should only be done for patients who are medically stable. Other patients may have a rapidly changing creatinine clearance, and therefore any result may be inaccurate.

eGFR: Formula-derived eGFR results have become widely used in clinical practice. The National Service Framework for Renal Services in the U.K. recommends the adoption of formula-derived eGFR in the annual evaluation of all patients with diabetes[22]. It is anticipated that this process will aid early identification and therefore improve longterm outcomes for those with diabetic nephropathy.

The American Diabetes Association recommends estimation of glomerular filtration rate by eGFR (in millilitres per min per 1.73 m^2), which is calculated by the Cockcroft-Gault (CG) formula[23], corrected for Body Surface Area (BSA), and the Modification of Diet in Renal Disease (MDRD)[24] equation in all patients with diabetes. (1) CG derived eGFR: A simpler method for estimating creatinine clearance is based upon a formula (the Cockcroft-Gault formula) that includes a person's age, gender, weight, and serum creatinine level, but does not require the collection of a 24 h urine sample. The CG formula is[23] as follows: $eGFR = [140 - age\ (years)] \times weight\ (kg) \times k \times c / serum\ creatinine\ (\mu mol/L)$, (k is 1.23 for men and 1.04 for women and c adjusts for BSA[25]. c = 1.73/BSA, with BSA calculated using the following DuBois[26] formula), {BSA (m^2) = [weight (kg)] 0.425 × [height (cm)] 0.725 × 0.007184}; (2) MDRD derived eGFR: Renal function can be assessed by serum creatinine and eGFR and calculated using the abbreviated MDRD formula as follows[24]: $eGFR = 186 \times (SCR \times 0.011)^{-1.154} \times (age)^{-0.203} \times (0.742,\ if\ female) \times (1.210\ if\ African\ American)$ (SCR was serum creatinine expressed as μmol/L). Renal function has been graded according to the Kidney Disease Outcomes Quality Initiative guidelines: stage 1, ⩾ 90 mL/min per 1.73 m^2; stage 2, 60-89 mL/min per 1.73 m^2; stage 3, 30-59 mL/min per 1.73 m^2; stage 4, 15-29 mL/min per 1.73 m^2; and stage 5, < 15 mL/min per 1.73 m^2 (gf4-13); (3) Reexpressed MDRD equation: As significant error is introduced in the MDRD equation by use of different creatinine assays or calibration methods, the simplified MDRD was recently recalculated with serum creatinine measurements calibrated to an enzymatic assay[27]: $eGFR = 175 \times [serum\ creatinine\ (mg/dL)]^{-1.154} \times (years)^{-0.203} \times 0.742\ (if\ female) \times 1.212\ (if\ African\ American)$; (4) MCQ equation: A new equation was developed by Rule *et al*[28] for GFR estimation in chronic kidney disease patients and in healthy persons for the diagnosis of chronic kidney disease. This is expressed as: $(1.911 + 5.249/SCr - 2.114/SCr^2) - [0.00686 \times age\ (years)] - 0.205$ if female, where SCr is serum creatinine [in milligrams per deciliter]; and (5) Considerations: eGFR is used for assessment of kidney function in patients with diabetes. However, despite validation in chronic kidney disease, eGFR has limitations in patients with preserved kidney function. These equations do have recognized limitations, including a tendency to significantly underestimate higher levels of GFR[29]. Additionally, Parving and colleagues[30] demonstrated that in type 2 diabetic subjects with macroalbuminuria, eGFR had a poor sensitivity for GFR values < 60 mL/min per 1.73 m^2. In obese patients with established kidney disease, the Cockcroft-Gault equation overestimates GFR while underestimating GFR in lean subjects, possibly due to increasing weight; while performance of the MDRD equation in such patients is consistent regardless of weight. Bias of the Cockcroft-Gault formula was most pronounced in lean subjects, diminishing with increasing body weight. Conversely, bias of the MDRD equation increased with increasing body weight[31]. In obese patients, excess body weight is mainly adipose

tissue, whereas creatinine is primarily generated by muscle. In the Cockcroft-Gault equation, body weight is proportional to GFR; therefore, increasing body weight without a proportional increase in creatinine generation will tend to increase the estimation of GFR. However, weight is not included in the MDRD equation and therefore cannot influence performance.

There are reports[32,33] that variation in calibration of the creatinine assay has an adverse impact on the performance of eGFR to estimate GFR, particularly at low levels of serum creatinine.

Cystatin-C based eGFR

A large percentage of individuals with type 2 diabetes pass through a period of pre-diabetes and may experience early renal dysfunction, e.g. a GFR > 60 mL/min per 1.73 m^2. Serum creatinine has been found to be defecient to detect mild renal impairment, even when used with prediction equations[34,35]. So, interest has developed in Cystatin-C, a non-glycosylated basic protein, as a potential endogenous filtration marker of GFR. Cystatin-C is a cysteine protease inhibitor that is produced by virtually all nucleated cells and released into the bloodstream. It is entirely filtered by the kidney glomerulus and metabolized by the proximal tubule[36].

Various formulae have been used to measure serum cystatin levels by different methodologies. Recent estimations were done using a particle-enhanced immunonephelometric[37] assay or immunoturbidimetric assays. In all formulae, Cys C is serum cystatin-C (in milligrams per liter).

Arnal *et al*[38] estimated eGFR in 208 patients aged 1-80 years with various etiologies as follows: Cys-eGFR (Arnal-Dade) = 74.835/(Cys C1.333). Rule *et al*[39] studied native kidney disease (gf7-15) patients (n = 204) having hypertension as suspected etiology: Cys-eGFR (Rule) = 66.8 - (Cys C) - 1.30. Isotopic GFR (iGFR) was measured by iothalamate clearance. MacIsaac *et al*[40] studied 126 diabetic patients (mainly type 2 diabetes). The iGFR was measured by clearance of 99mTc-diethylene-triamine-penta-acetic acid. The equation is as follows: Cys-eGFR (MacIsaac) = (84.6/Cys C) - 3.2. Tan *et al*[41] used an unbiased conversion algorithm between plasma cystatin-C and iGFR measured by iohexol clearance in type 1 diabetes, including a subgroup of healthy subjects as follows: Cys-eGFR (Tan) = (87.1/plasma Cys C) - 6.87. Erosha *et al*[37] measured GFR using the equation Cys-GFR (Erosha) = (86.7/Cys C) - 4.2. The intra- and interassay coefficient of variation for Cystatin-C were 2.58 and 3.95%, respectively, at a concentration of 1.54 mg/L.

Considerations: It has been shown that Cystatin-C is a more sensitive indicator of mild renal impairment and may better estimate the GFR than serum creatinine[42]. Moreover, concentrations of Cystatin-C are not affected by sex, age, or muscle mass[43]. There is supportive evidence that the reciprocal of Cystatin-C correlates more closely with isotopic GFR than the CG or MDRD equations in subjects with mild renal impairment[44]. As the identification of those with pre-diabetes is assuming greater importance, Cystatin-C may play a role in detection of the association between renal and heart disease in etiology of pre-diabetics. Recent clinical trials[45,46] among people with pre-diabetes have provided convincing evidence that early intervention can significantly delay or prevent the progression to type 2 diabetes. However, concerns remain regarding intrapatient variation and the effect of certain drugs and hormonal levels on Cystatin-C concentration[47].

Microalbumin (urine)

Albuminuria is a well-known predictor of poor renal outcomes in patients with type 2 diabetes and in essential hypertension[48,49]. Albuminuria has also been shown to be a predictor of cardiovascular outcomes in these populations. There is emerging data that reduction of albuminuria leads to reduced risk of adverse renal and cardiovascular events[50,51]. It has become increasingly clear that albuminuria should not only be measured in all patients with type 2 diabetes and hypertension, but also that steps should be taken to suppress albuminuria in order to prevent future renal and cardiovascular adverse events. Albuminuria may reflect underlying renal expression of vascular damage, hypertension, endothelial dysfunction[12], and low-grade inflammation[52].

Expected results: Microalbuminuria is defined as levels of albumin ranging from 30 to 300 mg in a 24 h urine collection[53]. Overt albuminuria, macroalbuminuria, or proteinuria is defined as a urinary albumin excretion of > 300 mg/24 h. Urinary albuminuria comprises 20%-70% or urinary total protein excretion. Albuminuria can be measured in several ways (Table 2): (1) measurement of albumin-to creatinine ratio in a random or first morning spot collection; (2) 24 h urine collection with measurement of creatinine to verify adequacy of the collection; and (3) timed (4 h or overnight) urine collections.

Considerations: South Asians are very prone to obesity and type 2 diabetes. This explains their susceptibility for central obesity and insulin resistance. It also indicates the higher rates of end-stage diabetic nephropathy in migrant South Asians[54]. They have a three times higher risk of developing diabetic nephropathy and an almost 40-fold increased risk for end-stage diabetic nephropathy when compared with Caucasians[55]. Prataap K *et al*[56] shows that central obesity is an early and independent risk factor for increased albuminuria in normoglycemic South Asian subjects.

Recently, Michiaki Fukui *et al*[57] showed the association of serum bilirubin level with microalbuminuria and subclinical atherosclerosis in patients with type 2 diabetes. Serum bilirubin concentrations were significantly lower in patients with cardiovascular disease (CVD). They were an independent determinant of CVD and had a significant inverse correlation to the log urinary albumin excretion.

Table 2 Expected results for microalbuminuria

Tests	Normal	Microalbuminuria	Macroalbuminuria
24 hr protein (mg)	< 150	< 500	⩾ 500
24 hr albumin (mg)	< 30	30-300	> 300
Timed collection for albumin (μg/min)	< 20	20-200	> 200
Spot sample collection for albumin (μg albumin/mg creatinine)	< 30	30-300	> 300

PROSPECTIVE FUTURE MARKERS

Several biochemical markers have the potential to be markers of CKD progression. These parameters might reflect diminished glomerular filtration, disturbances in tubular function or unknown contributors to kidney function that are unrelated to glomerular or tubular function. The evidence is still too sparse for most of these markers to be recommended for broad clinical use in the diagnosis of CKD progression. The emerging parameters associated with CKD progression must be studied further to determine whether they are causally related to progression of CKD or whether they simply predict the probability of progression. Few of these are as follows.

Retinol-binding protein 4

Retinol-binding protein 4 (RBP4) is a small visceral protein (approximately 21 kDa), mainly synthesized in the liver and catabolized in the kidneys after glomerular filtration[58]. RBP4 is complexed by transthyretin before delivering its ligand retinol to the target tissues[59]. RBP 4 was initially reported as an adipokine that impairs insulin sensitivity. The concentrations of this adipokine were found to increased in human subjects with impaired glucose tolerance (IGT) and type 2 diabetes compared with normal glucose tolerance subjects[60].

In prior studies, urinary RBP 4 excretion is found to be increased in early diabetic nephropathy and might even be a marker of early renal damage preceding microalbuminuria[61]. Andrea Henze *et al*[62] evaluated the influence of eGFR on RBP4 level and found that gradual elevation of RBP4 serum levels was accompanied by decline in eGFR in both, type 2 diabetic and non-diabetic subjects. No influence of type 2 diabetes mellitus or other parameters of diabetes (HbA1c, fasting serum glucose, BMI) on RBP4 serum concentration was seen. The association of RBP4 with several other metabolic parameters has been studied but limited studies are available on the relationship between this adipokine and mild to moderate decrease in GFR.

Adiponectin

Adiponectin is a recently discovered 30 kDa protein exclu sively secreted by adipocytes and is present at concen trations of 5-30 μg/mL in healthy humans. It is consid ered to be an important modulator of insulin sensitivity, dyslipidemia with anti-inflammatory properties[63,64].

Julie Lin *et al*[65] found the inverse correlation of serum adiponectin with the presence of renal dysfunction in men with type 2 diabetes, the majority of whom had well-preserved eGFR (87% had eGFR > 60 mL/min per 1.73 m^2).Adiponectin was 2.5 times higher in hemodialysis patients (15.0 *vs* 6.3 μg/mL, $P < 0.0001$)[66] and three times higher in pediatric peritoneal dialysis patients when compared with healthy control subjects.

Connective tissue growth factor

Connective tissue growth factor (CTGF) is a 36 to 38 kDa polypeptide with functions in extracellular matrix production and other profibrotic activity mediated by transforming growth factor-β1. Other biological functions of CTGF include angiogenesis, chondrogenesis, osteogenesis, and cell adhesion, migration, proliferation, and differentiation[67]. The upregulation of CTGF has been observed in human and experimental diabetic nephropathy[68].

Nguyen *et al*[69] revealed that urinary CTGF excretion is associated with urinary albumin excretion and associated inversely with glomerular filtration rate, both important clinical markers for severity of renal disease. Further, they have shown that[70] the plasma CTGF level correlates with rate of decline in GFR and that it is an independent predictor of both ESRD and mortality in patients with type 1 diabetic nephropathy. Baseline plasma CTGF was higher in patients with diabetic nephropathy than in patients with normoalbuminuria.

α1-microglobulin

α1-microglobulin is a 26 000-31 000 Da glycoprotein which exists in blood as a free form and or complexed with IgA and albumin. Because of its low molecular weight, the free form is filtered freely through the renal glomerular basement membrane and reabsorbed by the proximal tubular cells[71]. Hence, any proximal tubular cell dysfunction results in increased quantities of α1-microglobulin in the urine.

Urinary α1- microglobulin levels were found to be elevated in both type 1 and type 2 diabetic subjects. In type 2 diabetic subjects, α1-microglobulin excretion was directly correlated with albuminuria and HbA1c levels, and was decreased with improved glycemic control in causacians[72,73]. Similar findings have been shown in Asian population[74].

Transforming growth factor-β

Transforming growth factor (TGF)-β1 has a central role in fibrotic kidney disease and interstitial fibrosis (5). In type 2 diabetic patients, urinary TGF-β1 is elevated[75] and associated with histologically-proven severe mesangial expansion[76]. Although urinary TGF-β1 measurement has been suggested as a marker for diabetic nephropathy, not all studies have shown the association of urinary TGF-β1 with diabetic nephropathy. Eija *et al*[77] did not

find a difference in urinary TGF-β1 excretion between microalbuminuric and normoalbuminuric patients and only weak correlation was found with urinary glucose and β1-microglobulin. The association between urinary TGF-β1 and diabetic nephropathy may not be a direct one.

Liver-type fatty acid binding protein (L-FABP)

This protein is expressed in proximal tubular cells[78]. It has been shown that urinary L-FABP (U-LFABP) excretion is strongly associated with structural and functional tubular kidney damage in diabetic nephropathy[79]. Suzuki *et al*[80] performed a cross-sectional study in 356 adult type 2 diabetic patients and found a significant association between the stage of diabetic nephropathy and U-LFABP, although no significant difference between the normoalbuminuric and microalbuminuric groups was seen. Stine *et al*[81] have shown that U-LFABP, is elevated in type 1 diabetic patients compared with nondiabetic healthy control subjects and that the level further increases with micro and macroalbuminuria, reflecting increased tubular damage. There were no significant correlations between U-LFABP and sex, age, or A1C. Large studies with long-term follow-up are required to confirm these findings.

CONCLUSION

Diabetic nephropathy, especially related to type 2 diabetes, has become the single most important cause of ESRD worldwide. Management of traditional risk factors such as hypertension, hyperlipidemia, and smoking to improve cardiovascular and renal outcomes continues to be important in patients with chronic kidney disease. There is, however, growing recognition that nontraditional risk factors such as increased urinary albumin excretion, hypoalbuminemia, elevated serum creatinine levels, and/or decreased haemoglobin levels may also be important in individuals with chronic kidney disease. The RENAAL risk score for ESRD emphasizes the importance of the identification of levels of albuminuria and hypoalbuminemia as well as increased serum creatinine, and decreased haemoglobin levels to predict the development of ESRD in patients with type 2 diabetes and nephropathy. Albuminuria is a known strong predictor for ESRD, but the contribution of serum albumin, serum creatinine, and hemoglobin level further enhances the prediction of ESRD[82].

REFERENCES

1 **King H**, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. *Diabetes Care* 1998; **21**: 1414-1431
2 **Chowdhury TA**, Lasker SS. Complications and cardiovascular risk factors in South Asians and Europeans with early-onset type 2 diabetes. *QJM* 2002; **95**: 241-246
3 United States Renal Data System. *USRDS 2007 Annual Data Report*. Bethesda, MD: National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, U.S. Department of Health and Human Services, 2007
4 National Institute of Diabetes and Digestive and Kidney Diseases. *National Diabetes Statistics, 2007*. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2008
5 **Ramachandran A**. Epidemiology of diabetes in India--three decades of research. *J Assoc Physicians India* 2005; **53**: 34-38
6 Ramachandran A. Socio-economic burden of diabetes in India. *J Assoc Physicians India* 2007; **55** Suppl: 9-12
7 **Daneman D**. Early Diabetes-Related Complications in Adolescents. *Horm Res* 2005; **63**: 75-85
8 **Klag MJ**, Whelton PK, Randall BL, Neaton JD, Brancati FL, Ford CE, Shulman NB, Stamler J. Blood pressure and end-stage renal disease in men. *N Engl J Med* 1996; **334**: 13-18
9 **Mohan V**, Sandeep S, Deepa R, Shah B, Varghese C. Epidemiology of type 2 diabetes: Indian scenario. *Indian J Med Res* 2007; **125**: 217-230
10 **Go AS**, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; **351**: 1296-1305
11 **Bloomgarden ZT**. Diabetic nephropathy. *Diabetes Care* 2008; **31**: 823-827
12 **Stehouwer CD**, Henry RM, Dekker JM, Nijpels G, Heine RJ, Bouter LM. Microalbuminuria is associated with impaired brachial artery, flow-mediated vasodilation in elderly individuals without and with diabetes: further evidence for a link between microalbuminuria and endothelial dysfunction--the Hoorn Study. *Kidney Int Suppl* 2004; S42- S44
13 **Coresh J**, Astor BC, Greene T, Eknoyan G, Levey AS. Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third National Health and Nutrition Examination Survey. *Am J Kidney Dis* 2003; **41**: 1-12
14 **Hill JM**, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, Finkel T. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003; **348**: 593-600
15 **Sowers JR**. Hypertension, angiotensin II, and oxidative stress. *N Engl J Med* 2002; **346**: 1999-2001
16 **Heilig CW**, Brosius FC 3rd, Cunningham C. Role for GLUT1 in diabetic glomerulosclerosis. *Expert Rev Mol Med* 2006; **8**: 1-18
17 **Molitoris BA**. Acute kidney injury. In Goldman L, Ausiello D, editors. Cecil Medicine. 23rd ed. Philadelphia, Pa: Saunders Elsevier, 2007: chap 121
18 **Marti5n RF**. Renal Function. In Clin Chem Theory, Analysis, Correlation. 4th ed. Kaplan LA,Pesce AJ, Kazmierczak SC, editors. St. Louis, Missouri, Mosby, 2003: 483-484
19 **Harita N**, Hayashi T, Sato KK, Nakamura Y, Yoneda T, Endo G, Kambe H. Lower serum creatinine is a new risk factor of type 2 diabetes: the Kansai healthcare study. *Diabetes Care* 2009; **32**: 424-426
20 **DeFronzo RA**, Gunnarsson R, Björkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. *J Clin Invest* 1985; **76**: 149-155
21 **Bazari H**. Approach to the patient with renal disease. In Goldman L, Ausiello D, editors. Cecil Medicine. 23rd ed. Philadelphia, Pa: Saunders Elsevier, 2007: chap 115
22 Department of Health Renal Team. The National Service Framework for Renal Services. Part 2: chronic kidney disease, acute renal failure and end of life care, 2005. Available from: URL: http://www.kidney. org.uk/campaigns/Renal-nsf/nsf-pt2.pdf
23 **Cockcroft DW**, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; **16**: 31-41
24 **Levey AS, Bosch JP**, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; **130**: 461-470

25 **Chudleigh RA**, Dunseath G, Peter R, Harvey JN, Ollerton RL, Luzio S, Owens DR. Influence of body weight on the performance of glomerular filtration rate estimators in subjects with type 2 diabetes. *Diabetes Care* 2008; **31**: 47-49

26 **Du Bois D**, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. 1916. *Nutrition* 1989; **5**: 303-311; discussion 312-313

27 **Levey AS**, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, Van Lente F. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 2006; **145**: 247-254

28 **Rule AD**, Larson TS, Bergstralh EJ, Slezak JM, Jacobsen SJ, Cosio FG. Using serum creatinine to estimate glomerular filtration rate: accuracy in good health and in chronic kidney disease. *Ann Intern Med* 2004; **141**: 929-937

29 **Vervoort G**, Willems HL, Wetzels JF. Assessment of glomerular filtration rate in healthy subjects and normoalbuminuric diabetic patients: validity of a new (MDRD) prediction equation. *Nephrol Dial Transplant* 2002; **17**: 1909-1913

30 **Rossing P**, Rossing K, Gaede P, Pedersen O, Parving HH. Monitoring kidney function in type 2 diabetic patients with incipient and overt diabetic nephropathy. *Diabetes Care* 2006; **29**: 1024-1030

31 **Rigalleau V**, Lasseur C, Perlemoine C, Barthe N, Raffaitin C, Chauveau P, Combe C, Gin H. Cockcroft-Gault formula is biased by body weight in diabetic patients with renal impairment. *Metabolism* 2006; **55**: 108-112

32 **Coresh J**, Astor BC, McQuillan G, Kusek J, Greene T, Van Lente F, Levey AS. Calibration and random variation of the serum creatinine assay as critical elements of using equations to estimate glomerular filtration rate. *Am J Kidney Dis* 2002; **39**: 920-929

33 **Stevens LA**, Coresh J, Greene T, Levey AS. Assessing kidney function--measured and estimated glomerular filtration rate. *N Engl J Med* 2006; **354**: 2473-2483

34 **Poggio ED**, Wang X, Greene T, Van Lente F, Hall PM. Performance of the modification of diet in renal disease and Cockcroft-Gault equations in the estimation of GFR in health and in chronic kidney disease. *J Am Soc Nephrol* 2005; **16**: 459-466

35 **Nielsen S**, Rehling M, Schmitz A, Mogensen CE. Validity of rapid estimation of glomerular filtration rate in type 2 diabetic patients with normal renal function. *Nephrol Dial Transplant* 1999; **14**: 615-619

36 **Randers E**, Kristensen JH, Erlandsen EJ, Danielsen H. Serum Cystatin-C as a marker of the renal function. *Scand J Clin Lab Invest* 1998; **58**: 585-592

37 **Premaratne E**, MacIsaac RJ, Finch S, Panagiotopoulos S, Ekinci E, Jerums G. Serial measurements of Cystatin-C are more accurate than creatinine-based methods in detecting declining renal function in type 1 diabetes. *Diabetes Care* 2008; **31**: 971-973

38 **Thomas L**, Huber AR. Renal function--estimation of glomerular filtration rate. *Clin Chem Lab Med* 2006; **44**: 1295-1302

39 **Rule AD**, Bergstralh EJ, Slezak JM, Bergert J, Larson TS. Glomerular filtration rate estimated by Cystatin-C among different clinical presentations. *Kidney Int* 2006; **69**: 399-405

40 **Macisaac RJ**, Tsalamandris C, Thomas MC, Premaratne E, Panagiotopoulos S, Smith TJ, Poon A, Jenkins MA, Ratnaike SI, Power DA, Jerums G. Estimating glomerular filtration rate in diabetes: a comparison of cystatin-C- and creatinine-based methods. *Diabetologia* 2006; **49**: 1686-1689

41 **Tan GD**, Lewis AV, James TJ, Altmann P, Taylor RP, Levy JC. Clinical usefulness of Cystatin-C for the estimation of glomerular filtration rate in type 1 diabetes: reproducibility and accuracy compared with standard measures and iohexol clearance. *Diabetes Care* 2002; **25**: 2004-2009

42 **Sarnak MJ**, Katz R, Stehman-Breen CO, Fried LF, Jenny NS, Psaty BM, Newman AB, Siscovick D, Shlipak MG. Cystatin-C concentration as a risk factor for heart failure in older adults. *Ann Intern Med* 2005; **142**: 497-505

43 **Coll E**, Botey A, Alvarez L, Poch E, Quintó L, Saurina A, Vera M, Piera C, Darnell A. Serum Cystatin-C as a new marker for noninvasive estimation of glomerular filtration rate and as a marker for early renal impairment. *Am J Kidney Dis* 2000; **36**: 29-34

44 **Dharnidharka VR**, Kwon C, Stevens G. Serum Cystatin-C is superior to serum creatinine as a marker of kidney function: a meta-analysis. *Am J Kidney Dis* 2002; **40**: 221-226

45 **Knowler WC**, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002; **346**: 393-403

46 **Tuomilehto J**, Lindström J, Eriksson JG, Valle TT, Hämäläinen H, Ilanne-Parikka P, Keinänen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V, Uusitupa M. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001; **344**: 1343-1350

47 **Filler G**, Bökenkamp A, Hofmann W, Le Bricon T, Martínez-Brú C, Grubb A. Cystatin-C as a marker of GFR--history, indications, and future research. *Clin Biochem* 2005; **38**: 1-8

48 **Keane WF**, Brenner BM, de Zeeuw D, Grunfeld JP, McGill J, Mitch WE, Ribeiro AB, Shahinfar S, Simpson RL, Snapinn SM, Toto R. The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy: the RENAAL study. *Kidney Int* 2003; **63**: 1499-1507

49 **Pinto-Sietsma SJ**, Janssen WM, Hillege HL, Navis G, De Zeeuw D, De Jong PE. Urinary albumin excretion is associated with renal functional abnormalities in a nondiabetic population. *J Am Soc Nephrol* 2000; **11**: 1882-1888

50 **Brenner BM**, Cooper ME, de Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn SM, Zhang Z, Shahinfar S. Effects of losartan on renal and cardiovascular outcomes in patients with type 2 diabetes and nephropathy. *N Engl J Med* 2001; **345**: 861-869

51 **Ibsen H**, Olsen MH, Wachtell K, Borch-Johnsen K, Lindholm LH, Mogensen CE, Dahlöf B, Devereux RB, de Faire U, Fyhrquist F, Julius S, Kjeldsen SE, Lederballe-Pedersen O, Nieminen MS, Omvik P, Oparil S, Wan Y. Reduction in albuminuria translates to reduction in cardiovascular events in hypertensive patients: losartan intervention for endpoint reduction in hypertension study. *Hypertension* 2005; **45**: 198-202

52 **Ritz E**. Minor renal dysfunction: an emerging independent cardiovascular risk factor. *Heart* 2003; **89**: 963-964

53 Standards of medical care in diabetes. *Diabetes Care* 2005; **28** Suppl 1: S4-S36

54 **Mather HM**, Keen H. The Southall Diabetes Survey: prevalence of known diabetes in Asians and Europeans. *Br Med J (Clin Res Ed)* 1985; **291**: 1081-1084

55 **Chandie Shaw PK**, Baboe F, van Es LA, van der Vijver JC, van de Ree MA, de Jonge N, Rabelink TJ. South-Asian type 2 diabetic patients have higher incidence and faster progression of renal disease compared with Dutch-European diabetic patients. *Diabetes Care* 2006; **29**: 1383-1385

56 **Chandie Shaw PK**, Berger SP, Mallat M, Frölich M, Dekker FW, Rabelink TJ. Central obesity is an independent risk factor for albuminuria in nondiabetic South Asian subjects. *Diabetes Care* 2007; **30**: 1840-1844

57 **Fukui M**, Tanaka M, Shiraishi E, Harusato I, Hosoda H, Asano M, Hasegawa G, Nakamura N. Relationship between serum bilirubin and albuminuria in patients with type 2 diabetes. *Kidney Int* 2008; **74**: 1197-1201

58 **Goodman DS**. Plasma Retinol-Binding Protein. Orlando, Florida, New York Academic Press, Inc., 1984

59 **Blaner WS**. Retinol-binding protein: the serum transport protein for vitamin A. *Endocr Rev* 1989; **10**: 308-316

60 **Cho YM**, Youn BS, Lee H, Lee N, Min SS, Kwak SH, Lee HK, Park KS. Plasma retinol-binding protein-4 concentrations are

elevated in human subjects with impaired glucose tolerance and type 2 diabetes. *Diabetes Care* 2006; **29**: 2457-2461
61 **Abahusain MA**, Wright J, Dickerson JW, de Vol EB. Retinol, alpha-tocopherol and carotenoids in diabetes. *Eur J Clin Nutr* 1999; **53**: 630-635
62 **Henze A**, Frey SK, Raila J, Tepel M, Scholze A, Pfeiffer AF, Weickert MO, Spranger J, Schweigert FJ. Evidence that kidney function but not type 2 diabetes determines retinol-binding protein 4 serum levels. *Diabetes* 2008; **57**: 3323-3326
63 **Nedvídková J**, Smitka K, Kopský V, Hainer V. Adiponectin, an adipocyte-derived protein. *Physiol Res* 2005; **54**: 133-140
64 **Becker B**, Kronenberg F, Kielstein JT, Haller H, Morath C, Ritz E, Fliser D. Renal insulin resistance syndrome, adiponectin and cardiovascular events in patients with kidney disease: the mild and moderate kidney disease study. *J Am Soc Nephrol* 2005; **16**: 1091-1098
65 **Lin J**, Hu FB, Curhan G. Serum adiponectin and renal dysfunction in men with type 2 diabetes. *Diabetes Care* 2007; **30**: 239-244
66 **Zoccali C**, Mallamaci F, Tripepi G, Benedetto FA, Cutrupi S, Parlongo S, Malatino LS, Bonanno G, Seminara G, Rapisarda F, Fatuzzo P, Buemi M, Nicocia G, Tanaka S, Ouchi N, Kihara S, Funahashi T, Matsuzawa Y. Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. *J Am Soc Nephrol* 2002; **13**: 134-141
67 **Perbal B**. CCN proteins: multifunctional signalling regulators. *Lancet* 2004; **363**: 62-64
68 **Riser BL**, Denichilo M, Cortes P, Baker C, Grondin JM, Yee J, Narins RG. Regulation of connective tissue growth factor activity in cultured rat mesangial cells and its expression in experimental diabetic glomerulosclerosis. *J Am Soc Nephrol* 2000; **11**: 25-38
69 **Nguyen TQ**, Tarnow L, Andersen S, Hovind P, Parving HH, Goldschmeding R, van Nieuwenhoven FA. Urinary connective tissue growth factor excretion correlates with clinical markers of renal disease in a large population of type 1 diabetic patients with diabetic nephropathy. *Diabetes Care* 2006; **29**: 83-88
70 **Nguyen TQ**, Tarnow L, Jorsal A, Oliver N, Roestenberg P, Ito Y, Parving HH, Rossing P, van Nieuwenhoven FA, Goldschmeding R. Plasma connective tissue growth factor is an independent predictor of end-stage renal disease and mortality in type 1 diabetic nephropathy. *Diabetes Care* 2008; **31**: 1177-1182
71 **Ekstrom B**, Peterson PA, Berggard I. A urinary and plasma alpha1-glycoprotein of low molecular weight: isolation and some properties. *Biochem Biophys Res Commun* 1975; **65**: 1427-1433
72 **Hofmann W**, Guder WG. [Urinary proteins in patients with diabetes mellitus]. *Klin Wochenschr* 1989; **67** Suppl 17: 37-39
73 **Martin P**, Hampton KK, Walton C, Tindall H, Davies JA. Microproteinuria in type 2 diabetes mellitus from diagnosis. *Diabet Med* 1990; **7**: 315-318
74 **Hong CY**, Hughes K, Chia KS, Ng V, Ling SL. Urinary alpha1-microglobulin as a marker of nephropathy in type 2 diabetic Asian subjects in Singapore. *Diabetes Care* 2003; **26**: 338-342
75 **Sharma K**, Ziyadeh FN, Alzahabi B, McGowan TA, Kapoor S, Kurnik BR, Kurnik PB, Weisberg LS. Increased renal production of transforming growth factor-beta1 in patients with type II diabetes. *Diabetes* 1997; **46**: 854-859
76 **Sato H**, Iwano M, Akai Y, Kurioka H, Kubo A, Yamaguchi T, Hirata E, Kanauchi M, Dohi K. Increased excretion of urinary transforming growth factor beta 1 in patients with diabetic nephropathy. *Am J Nephrol* 1998; **18**: 490-494
77 **Korpinen E**, Teppo AM, Hukkanen L, Akerblom HK, Grönhagen-Riska C, Vaarala O. Urinary transforming growth factor-beta1 and alpha1-microglobulin in children and adolescents with type 1 diabetes. *Diabetes Care* 2000; **23**: 664-668
78 **Maatman RG**, van de Westerlo EM, van Kuppevelt TH, Veerkamp JH. Molecular identification of the liver- and the heart-type fatty acid-binding proteins in human and rat kidney. Use of the reverse transcriptase polymerase chain reaction. *Biochem J* 1992; **288** (Pt 1): 285-290
79 **Kamijo A**, Sugaya T, Hikawa A, Okada M, Okumura F, Yamanouchi M, Honda A, Okabe M, Fujino T, Hirata Y, Omata M, Kaneko R, Fujii H, Fukamizu A, Kimura K. Urinary excretion of fatty acid-binding protein reflects stress overload on the proximal tubules. *Am J Pathol* 2004; **165**: 1243-1255
80 **Suzuki K**, Babazono T, Murata H, Iwamoto Y. Clinical significance of urinary liver-type fatty acid-binding protein in patients with diabetic nephropathy. *Diabetes Care* 2005; **28**: 2038-2039
81 **Nielsen SE**, Sugaya T, Tarnow L, Lajer M, Schjoedt KJ, Astrup AS, Baba T, Parving HH, Rossing P. Tubular and glomerular injury in diabetes and the impact of ACE inhibition. *Diabetes Care* 2009; **32**: 1684-1688
82 **Keane WF**, Zhang Z, Lyle PA, Cooper ME, Zeeuw DD, Grunfeld JP. Risk Scores for Predicting Outcomes in Patients with Type 2 Diabetes and Nephropathy: The RENAAL Study. *Clin J Am Soc Nephrol* 2006; **10**: 1-7

S- Editor Zhang HN **L- Editor** Hughes D **E- Editor** Liu N

World Journal of Diabetes

Online Submissions: http://www.wjgnet.com/1948-9358office
wjd@wjgnet.com
doi:10.4239/wjd.v1.i2.57

World J Diabetes 2010 May 15; 1(2): 57-64
ISSN 1948-9358 (online)

REVIEW

Neurodegeneration: An early event of diabetic retinopathy

Marta Villarroel, Andreea Ciudin, Cristina Hernández, Rafael Simó

Marta Villarroel, Andreea Ciudin, Cristina Hernández, Rafael Simó, Diabetes and Metabolism Research Unit. Institut de Recerca Hospital Universitari Vall D'Hebron, Universitat Autònoma de Barcelona, Barcelona 08035, Spain
Marta Villarroel, Cristina Hernández, Rafael Simó, CIBER for Diabetes and Associated Metabolic Diseases (CIBERDEM, Instituto de Salut Carlos Ⅲ), Barcelona, Spain
Author contributions: Villarroel M and Ciudin A were responsible for the bibliographic research; Hernández C and Simó R prepared the first draft of the review; The final version of the paper was corrected and approved by all the authors.
Supported by Grants from the Ministerio de Ciencia e Innovacion, No. SAF2009-07408, CIBER de Diabetes y Enfermedades Metabólicas Asociadas and Generaltitat de Catalunya, No. 2009SGR739
Correspondence to: Rafael Simó, MD, PhD, Associate Professor, Diabetes Research Unit, Institut de Recerca Hospital Universitari Vall d'Hebron, Pg. Vall d'Hebron 119-129, Barcelona 08035, Spain. rsimo@ir.vhebron.net
Telephone: +34-93-4894172 Fax: +34-93-4894032
Received: October 9, 2009 Revised: March 3, 2010
Accepted: March 10, 2010
Published online: May 15, 2010

Abstract

Diabetic retinopathy (DR) has been classically considered to be a microcirculatory disease of the retina caused by the deleterious metabolic effects of hyperglycemia *per se* and the metabolic pathways triggered by hyperglycemia. However, retinal neurodegeneration is already present before any microcirculatory abnormalities can be detected in ophthalmoscopic examination. In other words, retinal neurodegeneration is an early event in the pathogenesis of DR which predates and participates in the microcirculatory abnormalities that occur in DR. Therefore, the study of the mechanisms that lead to neurodegeneration will be essential to identify new therapeutic targets in the early stages of DR. Elevated levels of glutamate and the overexpression of the renin- angiotensin-system play an essential role in the neurodegenerative process that occurs in diabetic retina. Among neuroprotective factors, pigment epithelial derived factor, somatostatin and erythropoietin seem to be the most relevant and these will be considered in this review. Nevertheless, it should be noted that the balance between neurotoxic and neuroprotective factors rather than levels of neurotoxic factors alone will determine the presence or absence of retinal neurodegeneration in the diabetic eye. New strategies, based on either the delivery of neuroprotective agents or the blockade of neurotoxic factors, are currently being tested in experimental models and in clinical pilot studies. Whether these novel therapies will eventually supplement or prevent the need for laser photocoagulation or vitrectomy awaits the results of additional clinical research.

Key words: Diabetic retinopathy; Angiotensin Ⅱ; Erythropoietin; Glutamate; Retinal neurodegeneration; Neuropeptides; Pigment epithelial derived factor; Somatostatin

Peer reviewers: Renu A Kowluru, PhD, Professor of Ophthalmology, Anatomy/Cell Biology, and Endocrinology, K404, Kresge Eye Institute, Wayne State University, 4717 St Antoine, Detroit, MI 48201, United States; Gregory I Liou, PhD, Professor, Department of Ophthalmology, Medical College of Georgia, Augusta, GA 30912-3400, United States

Villarroel M, Ciudin A, Hernández C, Simó R. Neurodegeneration: An early event of diabetic retinopathy. *World J Diabetes* 2010; 1(2): 57-64 Available from: URL: http://www.wjgnet.com/1948-9358/full/v1/i2/57.htm DOI: http://dx.doi.org/10.4239/wjd.v1.i2.57

INTRODUCTION

Diabetic retinopathy (DR) has been classically considered to be a microcirculatory disease of the retina due to the deleterious metabolic effects of hyperglycemia *per se* and the metabolic pathways triggered by hyperglycemia (polyol pathway, hexosamine pathway, DAG-PKC pathway, advanced glycation end-products and oxidative stress). However, retinal neurodegeneration is already

present before any microcirculatory abnormalities can be detected in ophthalmoscopic examination. In other words, retinal neurodegeneration is an early event in the pathogenesis of DR which predates and participates in the microcirculatory abnormalities that occur in DR[1,2]. This concept was first introduced by Barber *et al*[3]. These authors observed that one month after inducing diabetes in rats by using streptozotocin there was a high rate of apoptosis (TUNEL positive cells) in the neuroretina without a significant apoptosis in endothelial cells. In the same paper, the authors found a higher rate of apoptosis in the neuroretina from diabetic donors compared to non-diabetic donors, even in the case of a diabetic donor without microvascular abnormalities. These findings have been further confirmed in experimental models. In addition, it has been demonstrated in experimental models that, apart from apoptosis, another feature of retinal neurodegeneration is glial activation[1-5]. Our research group has been able to demonstrate that both apoptosis and glial activation occur in the retina of diabetic patients and precede microvascular abnormalities[6,7] (Figure 1).

Retinal ganglion cells are the earliest cells affected and have the highest rate of apoptosis[8]. However, an elevated rate of apoptosis has been also observed in the outer nuclear layer (photoreceptors)[9] and in the retinal pigment epithelium (RPE)[10].

Neuroretinal damage produces functional abnormalities such as the loss of chromatic discrimination, contrast sensitivity and dark adaptation. These alterations can be detected by means of electrophysiological studies in diabetic patients with diabetes duration of less than two years, i.e. before microvacular lesions can be detected in ophthalmologic examination. In addition, neuroretinal degeneration will initiate and/or activate several metabolic and signalling pathways which will participate in the microangiopathic process as well as in the disruption of the blood-retinal barrier (a crucial element in the pathogenesis of DR)[11].

The underlying mechanisms that lead to neuronal deficits are likely to be broad. In addition, it is unknown which of the two primordial pathological elements (apoptotis or glial activation) is the first to appear and is consequently the primary event. Nevertheless, it seems that retinal glial cells play an essential role in maintaining the normal function of the retina. When Müller cells (the principal glial cells in the retina) become gliotic, display altered potassium siphoning, glutamate and GABA uptake are also known to express several modulators of angiogenesis[12].

IN VIVO EXPERIMENTAL MODELS TO STUDY RETINAL NEURODEGENERATION IN THE SETTING OF DR

Since neurodegeneration is an early event in the pathogenesis of DR, it is not necessary to use animal models with microangiopatic lesions in the eye such as Torii or Goto-Kakizaki rats. The experimental model currently used to study retinal neurodegeneration in DR is the rat with streptozotocin-induced diabetes (STZ-DM). In this model, electroretinographic abnormalities are present two weeks after inducing diabetes[13] and the presence of neural apoptosis and glial reaction can be clearly detected one month after starting diabetes[3,5]. However, it should be noted that the interpretation of the results of retinal neurodegeneration in STZ-DM rats might be hampered by the neurotoxic effect of STZ. Streptozotocin is a potent neurotoxic agent and is able to produce neural degeneration. Therefore, neurodegeneration (apoptosis + glial activation) observed in rats with STZ-DM could be due to STZ itself rather than the metabolic pathways related to diabetes. It is worthy of mention that pathological changes to the brain after an intraventricular injection of STZ are very similar to the neurodegeneration reported in DR[14]. Therefore, it may be advisable to use murine models with a spontaneous development of diabetes or at least experimental models in which diabetes has not been induced by a neurotoxic drug.

Mice have been used much less than rats as experimental models for the study of DR and retinal neurodegeneration. This is because they are more resistant to the STZ effect (mice need 3-5 doses of STZ to induce diabetes whereas in rats one dose is sufficient), have lower eye cups and present a lower degree of lesions compared to rats. This relative protection in developing pathological lesions related to diabetes can be partly attributed to a lower activity of aldose reductase (polyol pathway) compared to rats[5]. Nevertheless, because of its great potential for genetic manipulation, the mouse offers a unique opportunity to study the molecular pathways involved in disease development. Among mice, C57BL/KsJ-*db*/*db* is the model that best reproduces the neurodegenerative features observed in patients with DR. C57BL/KsJ-*db*/*db* mice carry a mutation in the leptin receptor gene and are a model for obesity-induced type 2 diabetes. They develop hyperglycemia starting at ~8 wk of age as a result of excessive food consumption. It is noteworthy that they present an abundant expression of aldose-reductase in the retina (an important differential trait from other mouse models)[15]. Therefore, C57BL/KsJ-*db*/*db* seems appropriate for investigating the underlying mechanisms of retinal neurodegeneration associated with diabetes and for testing new drugs.

NEUROTOXIC AND NEUROPROTECTIVE FACTORS

The main neurotoxic metabolite involved in diabetic retinal neurodegeneration is glutamate. In addition, there is emerging information about the neurotoxicity due to angiotensin II in the setting of the RAS overexpression that exists in DR. The role of other neurotoxic factors has yet to be elucidated.

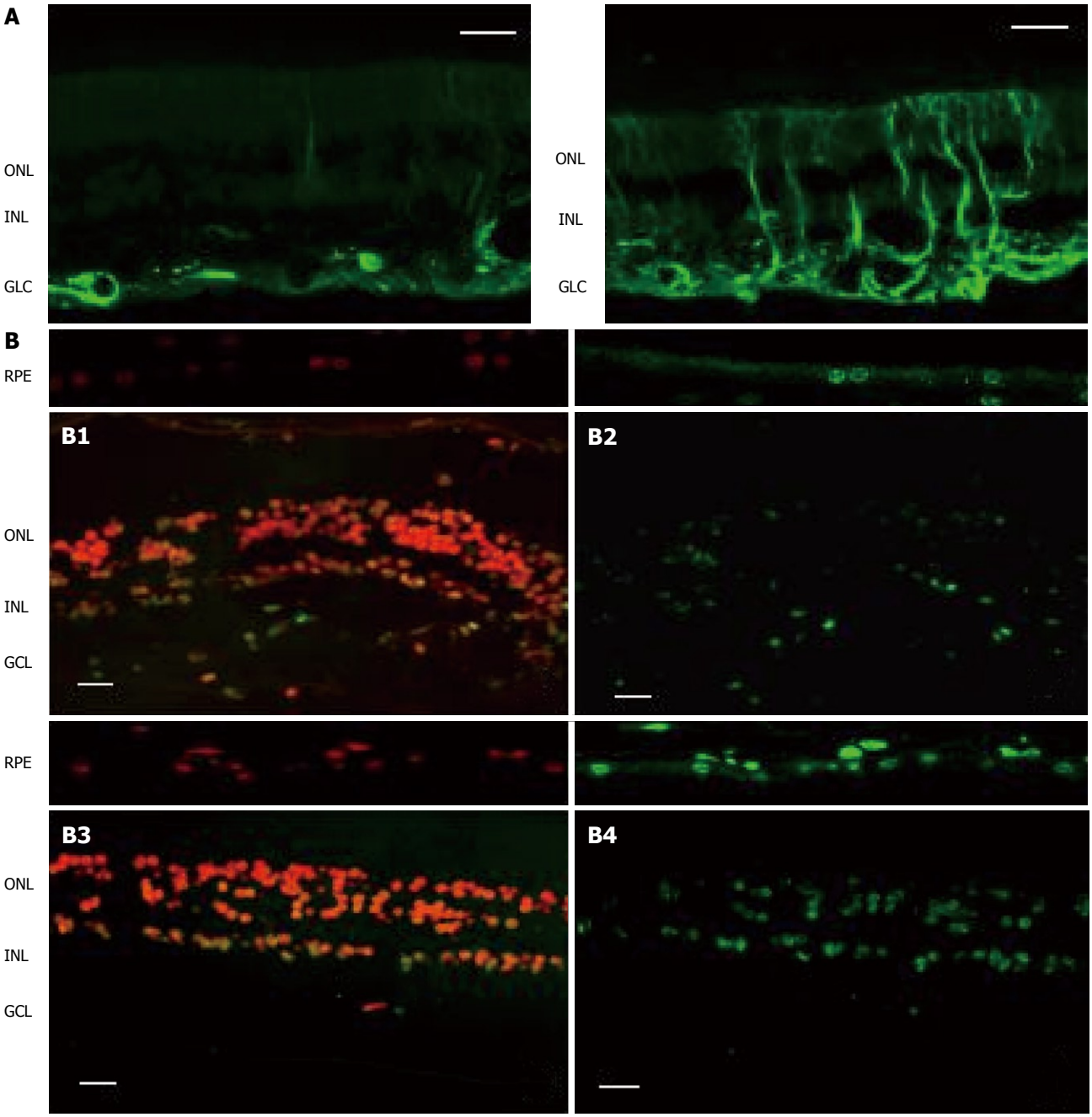


Figure 1 Comparison of the two key elements of neurodegeneration (glial activation and apoptosis) between a representative case of diabetic patient without DR and a non-diabetic subject. As can be seen, neurodegeneration is higher in the retina from the diabetic donor. A: Glial activation in the human retina. Glial fibrillar acidic protein (GFAP) immunofluorescence (green) from a non-diabetic donor (left panel) and a diabetic donor (right panel); B: Apoptosis in the human retina. Upper panel: Non-diabetic donor (B1: Propidium iodide, B2: TUNEL immunofluorescence). Low panel: Diabetic donor (B3: Propidium iodide, B4: TUNEL immunofluorescence). RPE: Retinal pigment epithelium; ONL: Outer nuclear layer; INL: Inner nuclear layer; GCL: Ganglion cell layer. The bar represents 20 μm.

Several types of insult cause the upregulation of neurotrophic factors and their receptors in the retina resulting in decreased photoreceptor cell death from subsequent injury. This phenomenon is more prominent in rats than in mice and neurotrophic factors are more efficacious in rats than mice[16]. Among the neuroprotective factors, pigment epithelial derived factor (PEDF), somatostatin (SST) and erythropoietin (Epo) seem to be the most relevant and will be reviewed in this paper. However, there are other neuroprotective factors such as neuroprotectin D1 (NPD1), brain-derived neurotrophic factor (BDNF), glial cell-line-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF) and adrenomedullin (AM). It is worthy of mention that the balance between the neurotoxic and neuroprotective factors will determine the fate of the retinal neurons.

Glutamate

Glutamate is the major excitatory neurotransmitter in the retina and is involved in neurotransmission from photoreceptors to bipolar cells and from bipolar cells to ganglion cells. However, an elevated glutamate level (which

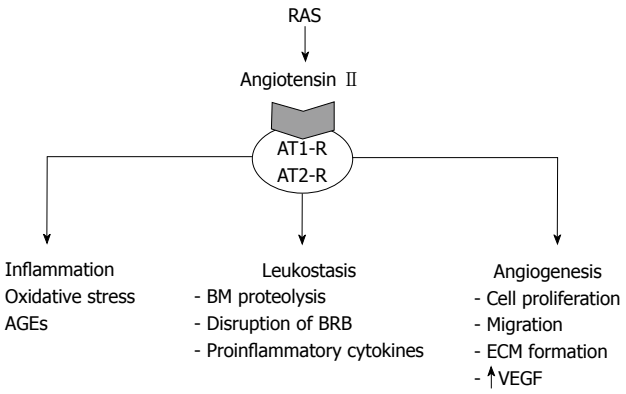


Figure 2 AT1-R activation by angiotensin Ⅱ produced by the retina stimulates several pathways involved in the pathogenesis of DR such as inflammation, oxidative stress, leukostasis and angiogenesis. AT1-R: Angiotensin Ⅱ type 1 receptor; AT2-R: Angiotensin Ⅱ type 2 receptor; AGEs: Advanced glycated end products; BM: Basal membrane; BRB: Blood retinal barrier; ECM: Extracellular matrix; VEGF: Vascular endothelial growth factor.

results in excessive stimulation) is implicated in the so called "excitotoxicity" which leads to neurodegeneration. The excitotoxicity of glutamate is the result of over-activation of N-metil-D-aspartame receptors which have been overexpressed in DM-STZ rat receptors[17]. There are at least two mechanisms involved in glutamate-induced apoptosis: a caspase-3-dependent pathway and a caspase-independent pathway involving calpain and mitochondrial apoptosis inducing factor[18].

Elevated levels of glutamate in the retina have been found in experimental models of diabetes as well as in the vitreous fluid of diabetic patients with PDR[19,20]. However, there is no information about this issue in the earlier stages of DR.

The cause of the high levels of glutamate in DR has been related to a dysfunction of macroglia in metabolising glutamate[21]. The reason for this dysfunction seems to be related to impairment in the glutamate transporter of Müller cells due to diabetes-induced oxidative stress[22]. In addition, two enzymatic abnormalities in glutamate metabolism have been found in the diabetic retina: transamination to alpha-ketoglutarate and amination to glutamine. The reduced flux through these pathways may be associated with the accumulation of glutamate[23].

Angiotensin Ⅱ

The blockade of the RAS with a converting enzyme inhibitor or by using angiotensin Ⅱ type 1 (AT1) receptor blockers (ARBs) is one of the most used strategies for hypertension treatment in diabetic patients. Apart from the kidney, the RAS system is expressed in the eye. In the retina, RAS components are largely found and synthesized in two sites: neurons and glia cells in the inner retina and in blood vessels[24]. The finding of renin and angiotensin in glia and neurons suggests a role for these molecules in neuromodulation.

There is growing evidence that RAS activation in the eye plays an important role in the pathogenesis of DR[24]. Therefore, apart from lowering BP, the blockade of the RAS could also be beneficial "per se" in reducing the development and progression of DR. In fact, recent evidence supports the concept that RAS blockade in normotensive patients has beneficial effects in the incidence and progression of DR[25-27].

The major components of RAS have been identified in ocular tissues and are overexpressed in the diabetic retina. Angiotensin Ⅱ binds and activates two primary receptors, AT1-R and AT2-R. In adult humans activation of the AT1-R dominates the pathological states. AT1-R activation by angiotensin Ⅱ produced by the retina stimulates several pathways involved in the pathogenesis of DR such as inflammation, oxidative stress, cell proliferation, pericyte migration, remodelling of extracellular matrix by increasing matrix metalloproteinases, angiogenesis and fibrosis (Figure 2)[24]. In addition, AT1-R activation by angiotensin Ⅱ promotes leukostasis (the inappropriate adherence of leukocytes to the retinal capillaries) and neurodegeneration[24,28]. Apart from reducing microvascular disease, there is growing evidence pointing to neuroprotection as a relevant mechanism involved in the beneficial effects of ARBs in DR. In this regard, it has recently been reported that candesartan (the ARB with the best diffusion across the blood-brain barrier) has a neuroprotective effect after brain focal ischemia[29]. In addition, telmisartan and valsartan inhibit the synaptophysin degradation that exists in the retina of a murine model of DR[30]. Moreover, valsartan is able to prolong the survival of astrocytes and reduce glial activation in the retina of rats with hypoxia-induced retinopathy[31]. Furthermore, mitochondrial oxidative stress asociated with retinal neurodegeneration has been improved by using losartan in a model of spontaneously hypertensive rats[32]. Taken together, it seems that neuroprotection is a relevant mechanism involved in the beneficial effects of ARBs in DR.

PEDF

PEDF is a 50 kDa protein encoded by a single gene that is preserved across phyla from fish to mammals. It shares homology with the serine proteinase inhibitor (Serpin) family but lacks proteinase activity. PEDF was first purified from human RPE cells and was described as a neurotrophic factor with neuroprotective properties[33]. In this regard, it should be noted that intraocular gene transfer of PEDF significantly increases neuroretinal cell survival after ischemia-reperfusion injury[34] and excessive light exposure[35]. In addition, PEDF protects neurons from glutamate-mediated neurodegeneration[36].

Apart from its neurotrophic factor and neuroprotective properties, there is growing evidence that PEDF is among the most important natural inhibitors of angiogenesis and that it is the main factor accounting for the antiangiogenic activity of vitreous fluid where it is found in abundant quantities[37]. PEDF is responsible for the avascularity of the cornea and vitreous fluid and under hypoxic conditions its secretion is decreased. In addition, elevated glucose down-regulates PEDF expression in

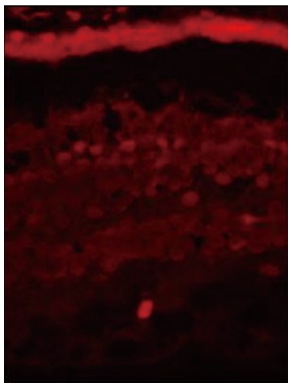


Figure 3 SST immunofluorescence (red) in the human retina showing a higher expression in RPE than in the neuroretina from a human eye donor. RPE: Retinal pigment epithelium; ONL: Outer nuclear layer; INL: Inner nuclear layer; GCL: Ganglion cell layer.

RPE cells. The receptors for PEDF are not known but experimental studies suggest that there may be receptors in the retina[38-40]. In addition, it has been suggested that antiangiogenic and neurotrophic activities reside in separate regions of the molecule, thus suggesting that more than one receptor exists[40,41].

Therefore, there are enough arguments to propose PEDF as a serious new candidate for DR treatment. PEDF can successfully be delivered to the eye by viral vectors. As an alternative to viral-mediated gene transfer, transplantation of autologous cells transfected with plasmids encoding for PEDF delivers therapeutic doses of PEDF to the eye. Another mechanism for delivering PEDF to the eye is to exploit its endogenous availability or production. It seems likely that much of the endogenous PEDF in the eye is bound to extracellular matrix molecules and thus may not be active. Drugs that release PEDF from these matrix molecules could increase free PEDF to therapeutic levels. In addition, levels of PEDF mRNA and secreted protein could be increased by either dexamethasone or retinoic acid[42]. Therefore, new strategies for diabetic retinopathy treatment based on PEDF activation are warranted.

SST

SST is a peptide that was originally identified as the hypothalamic factor responsible for the inhibition of the release of the growth hormone from the anterior pituitary. Subsequent studies have shown that SST has a much broader spectrum of inhibitory actions and is much more widely distributed in the body, occurring not only in many regions of the central nervous system but also in many tissues of the digestive tract and in the retina[43]. SST mediates its multiple biological effects via specific plasma membrane receptors that belong to the family of G-protein coupled receptors with seven transmembrane domains. So far, five SST receptor subtypes (SSTRs) have been identified (SSTRs 1-5).

Neuroretina and, in particular, the amacrine cells have been classically described as the main source of SST in the retina. However, we have found that SST expression and content is higher in RPE than in the neuroretina from human eye donors[6] (Figure 3). Therefore, RPE rather than neuroretina is the main source of SST, at least in humans. The amount of SST produced by the human retina is significant as deduced by the strikingly high levels found in the vitreous fluid[44,45]. Apart from SST, SSTRs are also expressed in the retina, with SSTR1 and SSTR2 being the most widely expressed[43,46,47]. The production of both SST and its receptors simultaneously suggests an autocrine action in the human retina.

The main functions of SST for retinal homeostasis are the following: (1) SST acts as a neuromodulator through multiple pathways, including intracellular Ca^{2+} signaling, nitric oxide function and glutamate release from the photoreceptors. In addition, a loss of SST immunoreactivity occurs after degeneration of the ganglion cells. Therefore, the neuroretinal damage that occurs in DR might be the reason for the decreased SST levels detected in the vitreous fluid of these patients. In fact, we have recently found that low SST expression and production is an early event in DR and is associated with retinal neurodegeneration (apoptosis and glial activation)[6]; (2) SST is a potent angiostatic factor. SST may reduce endothelial cell proliferation and neovascularisation by multiple mechanisms including the inhibition of postreceptor signalling events of peptide growth factors such as IGF-I, VEGF, epidermal growth factor and PDGF[48]; and (3) SST has been involved in the transport of water and ions. Various ion/water transport systems are located on the apical side of the RPE adjacent to the subretinal space and a high expression of SST-2 has been shown in this apical membrane of the RPE[46].

In DR there is a downregulation of SST associated with retinal neurodegeneration[6]. The lower expression of SST in RPE and neuroretina is associated with a dramatic decrease of intravitreal SST levels in both PDR[39,40] and DME[49]. As a result, the physiological role of SST in preventing both neovascularisation and fluid accumulation within the retina could be reduced and consequently the development of PDR and DME is favoured. In addition, the loss of neuromodulator activity could also contribute to neuroretinal damage. For all these reasons, intravitreal injection of SST analogues or gene therapy has been proposed as a new therapeutic approach in DR[50].

Epo

Erythropoietin (Epo) was first described as a glycoprotein produced exclusively in fetal liver and adult kidney that acts as a major regulator of erythropoiesis. However, Epo expression has also been found in the human brain and in the human retina[51,52]. In recent years, we have demonstrated that both Epo and its receptor are expressed in the adult human retina[53]. Epo and EpoR mRNAs are significantly higher in RPE than in the neuroretina[53]. In addition, intravitreal levels of Epo are ~3.5 fold higher that those found in plasma[52]. The role of Epo in the retina remains to be elucidated but it seems that it has a potent neuroprotective effect[54,55].

Epo is upregulated in DR[52,53,56,57]. Epo overexpression has been found in both the RPE and neuroretina of diabetic eyes (Figure 4)[52,53]. This is in agreement with the elevated concentrations of Epo found in the vitreous

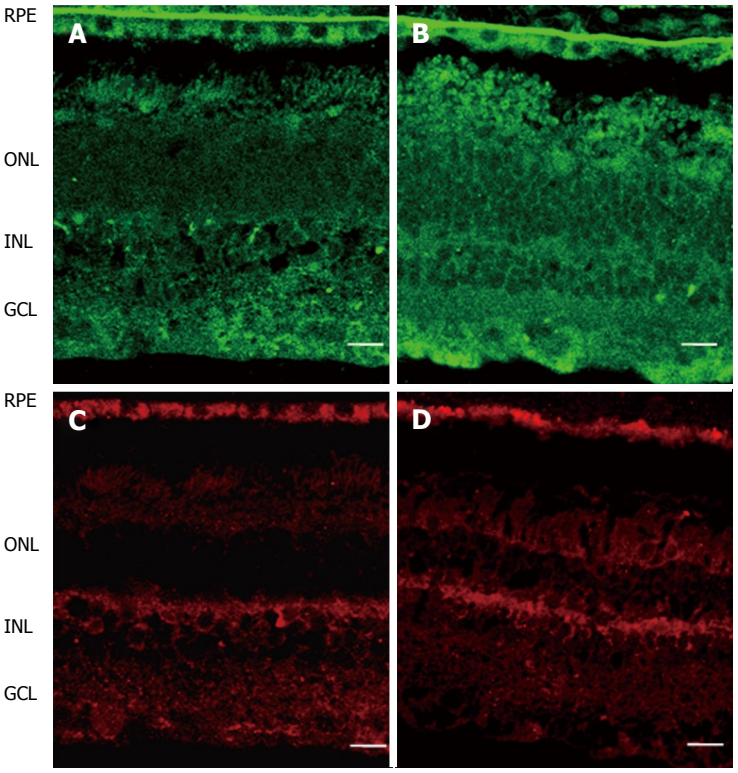


Figure 4 Comparison of Epo (upper pannel, green) and EpoR (lower pannel, red) immunofluorescence in the human retina between representative samples. A, C: Non-diabetic donor; B, D: Diabetic donor. RPE: Retinal pigment epithelium; ONL: Outer nuclear layer; INL: Inner nuclear layer; GCL: Ganglion cell layer. The bar represents 20 mm.

fluid of diabetic patients (~30 fold higher than plasma and ~10 fold higher than in non-diabetic subjects)[52]. Hypoxia is a major stimulus for both systemic and intraocular Epo production. In fact, high intravitreous levels of Epo have recently been reported in ischemic retinal diseases such as PDR[52,56-58]. In addition, it has been reported that Epo has an angiogenic potential equivalent to VEGF[57,59]. Therefore, Epo could be an important factor involved in stimulating retinal angiogenesis in PDR. However, intravitreal levels of Epo have been found at a similar range in PDR to that in DME (a condition in which hypoxia is not a predominant event)[52]. In addition, intravitreal Epo levels are not elevated in non-diabetic patients with macular edema secondary to retinal vein occlusion[60]. Finally, a higher expression of Epo has been detected in the retinas of diabetic donors at early stages of DR compared to non-diabetic donors and this overexpression is unrelated to mRNA expression of hypoxic inducible factors (HIF-1α and HIF-1β)[53]. Therefore, stimulating agents other than hypoxia/ischemia are involved in the upregulation of Epo that exists in the diabetic eye.

The reason why Epo is increased in DR remains to be elucidated but the bulk of the available information points to a protective effect rather than a pathogenic effect at least in the early stages of DR. In addition, Epo is a potent physiological stimulus for the mobilization of endothelial progenitor cells (EPCs) and therefore could play a relevant role in regulating the traffic of circulating EPCs towards injured retinal sites[61]. In this regard, the increase of intraocular synthesis of Epo that occurs in DR can be contemplated as a compensatory mechanism to restore the damage induced by the diabetic milieu. In fact, exogenous Epo administration by intravitreal injection in early diabetes may prevent retinal cell death and protect the blood retinal barrier function in STZ-DM rats[62]. Nevertheless, in advanced stages, the elevated levels of Epo could potentiate the effects of VEGF thus contributing to neovascularisation and consequently worsening PDR[61,63].

The potential advantages of Epo or EpoR agonists in the treatment of DR include neuroprotection, vessel stability and enhanced recruitment of EPCs to the pathological area. However, as mentioned above, timing is critical since if Epo is given at later hypoxic stages the severity of DR could increase. However, in the case of the eye, disease progression is easy to follow without invasive investigation and allows timing of the administration of drugs to be carefully monitored hopefully resulting in better clinical outcomes.

CONCLUSION

Neurodegeneration is an early event in the pathogenesis of DR. Elevated levels of glutamate and the overexpression of the RAS system play an essential role in the neurodegenerative process that occurs in the diabetic retina. Among the neuroprotective factors, PEDF, SST and Epo seem to be the most important contributing factors but the role of NPD1, BDNF, GDNF, CNTF and AM should also be taken into account. In fact, the balance between neurotoxic and neuroprotective factors rather than the levels of neurotoxic factors alone will determine the presence or not of retinal neurodegeneration in the diabetic eye.

Finally, it should be stressed that the study of the

mechanisms that lead to neurodegeneration will be essential for identifying new therapeutic targets in the early stages of DR. At present this is a growing and increasingly important field in medicine which involves several areas of knowledge such as diabetology, ophthalmology and the neurosciences and consequently clinical trials with a multidisciplinary approach are needed.

ACKNOWLEDGMENTS

M Villarroel is a recipient of a grant from the Fundació Institut de Recerca Hospital Universitari Vall d'Hebron. CIBER for Diabetes and Associated Metabolic Diseases is an initiative of the Instituto de Salud Carlos Ⅲ.

REFERENCES

1 **Barber AJ**. A new view of diabetic retinopathy: a neurodegenerative disease of the eye. *Prog Neuropsychopharmacol Biol Psychiatry* 2003; **27**: 283-2902
2 **Antonetti DA**, Barber AJ, Bronson SK, Freeman WM, Gardner TW, Jefferson LS, Kester M, Kimball SR, Krady JK, LaNoue KF, Norbury CC, Quinn PG, Sandirasegarane L, Simpson IA. Diabetic retinopathy: seeing beyond glucose-induced microvascular disease. *Diabetes* 2006; **55**: 2401-2411
3 **Barber AJ**, Lieth E, Khin SA, Antonetti DA, Buchanan AG, Gardner TW. Neural apoptosis in the retina during experimental and human diabetes. Early onset and effect of insulin. *J Clin Invest* 1998; **102**: 783-7916
4 **Lorenzi M**, Gerhardinger C. Early cellular and molecular changes induced by diabetes in the retina. *Diabetologia* 2001; **44**: 791-804
5 **Asnaghi V**, Gerhardinger C, Hoehn T, Adeboje A, Lorenzi M. A role for the polyol pathway in the early neuroretinal apoptosis and glial changes induced by diabetes in the rat. *Diabetes* 2003; **52**: 506-511
6 **Carrasco E**, Hernández C, Miralles A, Huguet P, Farrés J, Simó R. Lower somatostatin expression is an early event in diabetic retinopathy and is associated with retinal neurodegeneration. *Diabetes Care* 2007; **30**: 2902-2908
7 **Carrasco E**, Hernández C, de Torres I, Farrés J, Simó R. Lowered cortistatin expression is an early event in the human diabetic retina and is associated with apoptosis and glial activation. *Mol Vis* 2008; **14**: 1496-1502
8 **Kern TS**, Barber AJ. Retinal ganglion cells in diabetes. *J Physiol* 2008; **586**: 4401-4408
9 **Park SH**, Park JW, Park SJ, Kim KY, Chung JW, Chun MH, Oh SJ. Apoptotic death of photoreceptors in the streptozotocin-induced diabetic rat retina. *Diabetologia* 2003; **46**: 1260-1268
10 **Aizu Y**, Oyanagi K, Hu J, Nakagawa H. Degeneration of retinal neuronal processes and pigment epithelium in the early stage of the streptozotocin-diabetic rats. *Neuropathology* 2002; **22**: 161-170
11 **Tretiach M**, Madigan MC, Wen L, Gillies MC. Effect of Müller cell co-culture on in vitro permeability of bovine retinal vascular endothelium in normoxic and hypoxic conditions. *Neurosci Lett* 2005; **378**: 160-165
12 **Fletcher EL**, Phipps JA, Ward MM, Puthussery T, Wilkinson-Berka JL. Neuronal and glial cell abnormality as predictors of progression of diabetic retinopathy. *Curr Pharm Des* 2007; **13**: 2699-2712
13 **Li Q**, Zemel E, Miller B, Perlman I. Early retinal damage in experimental diabetes: electroretinographical and morphological observations. *Exp Eye Res* 2002; **74**: 615-625
14 **Shoham S**, Bejar C, Kovalev E, Schorer-Apelbaum D, Weinstock M. Ladostigil prevents gliosis, oxidative-nitrative stress and memory deficits induced by intracerebroventricular injection of streptozotocin in rats. *Neuropharmacology* 2007; **52**: 836-843
15 **Cheung AK**, Fung MK, Lo AC, Lam TT, So KF, Chung SS, Chung SK. Aldose reductase deficiency prevents diabetes-induced blood-retinal barrier breakdown, apoptosis, and glial reactivation in the retina of db/db mice. *Diabetes* 2005; **54**: 3119-3125
16 **Wahlin KJ**, Adler R, Zack DJ, Campochiaro PA. Neurotrophic signaling in normal and degenerating rodent retinas. *Exp Eye Res* 2001; **73**: 693-701
17 **Ng YK**, Zeng XX, Ling EA. Expression of glutamate receptors and calcium-binding proteins in the retina of streptozotocin-induced diabetic rats. *Brain Res* 2004; **1018**: 66-72
18 **Zhang Y**, Bhavnani BR. Glutamate-induced apoptosis in neuronal cells is mediated via caspase-dependent and independent mechanisms involving calpain and caspase-3 proteases as well as apoptosis inducing factor (AIF) and this process is inhibited by equine estrogens. *BMC Neurosci* 2006; **7**: 49
19 **Ambati J**, Chalam KV, Chawla DK, D'Angio CT, Guillet EG, Rose SJ, Vanderlinde RE, Ambati BK. Elevated gamma-aminobutyric acid, glutamate, and vascular endothelial growth factor levels in the vitreous of patients with proliferative diabetic retinopathy. *Arch Ophthalmol* 1997; **115**: 1161-1166
20 **Pulido JE**, Pulido JS, Erie JC, Arroyo J, Bertram K, Lu MJ, Shippy SA. A role for excitatory amino acids in diabetic eye disease. *Exp Diabetes Res* 2007; **2007**: 36150
21 **Lieth E**, Barber AJ, Xu B, Dice C, Ratz MJ, Tanase D, Strother JM. Glial reactivity and impaired glutamate metabolism in short-term experimental diabetic retinopathy. Penn State Retina Research Group. *Diabetes* 1998; **47**: 815-820
22 **Li Q**, Puro DG. Diabetes-induced dysfunction of the glutamate transporter in retinal Müller cells. *Invest Ophthalmol Vis Sci* 2002; **43**: 3109-3116
23 **Lieth E**, LaNoue KF, Antonetti DA, Ratz M. Diabetes reduces glutamate oxidation and glutamine synthesis in the retina. The Penn State Retina Research Group. *Exp Eye Res* 2000; **70**: 723-730
24 **Wilkinson-Berka JL**. Angiotensin and diabetic retinopathy. *Int J Biochem Cell Biol* 2006; **38**: 752-765
25 **Chaturvedi N**, Porta M, Klein R, Orchard T, Fuller J, Parving HH, Bilous R, Sjølie AK. Effect of candesartan on prevention (DIRECT-Prevent 1) and progression (DIRECT-Protect 1) of retinopathy in type 1 diabetes: randomised, placebo-controlled trials. *Lancet* 2008; **372**: 1394-1402
26 **Sjølie AK**, Klein R, Porta M, Orchard T, Fuller J, Parving HH, Bilous R, Chaturvedi N. Effect of candesartan on progression and regression of retinopathy in type 2 diabetes (DIRECT-Protect 2): a randomised placebo-controlled trial. *Lancet* 2008; **372**: 1385-1393
27 **Mauer M**, Zinman B, Gardiner R, Suissa S, Sinaiko A, Strand T, Drummond K, Donnelly S, Goodyer P, Gubler MC, Klein R. Renal and retinal effects of enalapril and losartan in type 1 diabetes. *N Engl J Med* 2009; **361**: 40-51
28 **Chen P**, Scicli GM, Guo M, Fenstermacher JD, Dahl D, Edwards PA, Scicli AG. Role of angiotensin II in retinal leukostasis in the diabetic rat. *Exp Eye Res* 2006; **83**: 1041-1051
29 **Krikov M**, Thone-Reineke C, Müller S, Villringer A, Unger T. Candesartan but not ramipril pretreatment improves outcome after stroke and stimulates neurotrophin BNDF/TrkB system in rats. *J Hypertens* 2008; **26**: 544-552
30 **Kurihara T**, Ozawa Y, Nagai N, Shinoda K, Noda K, Imamura Y, Tsubota K, Okano H, Oike Y, Ishida S. Angiotensin II type 1 receptor signaling contributes to synaptophysin degradation and neuronal dysfunction in the diabetic retina. *Diabetes* 2008; **57**: 2191-2198
31 **Downie LE**, Pianta MJ, Vingrys AJ, Wilkinson-Berka JL, Fletcher EL. AT1 receptor inhibition prevents astrocyte

degeneration and restores vascular growth in oxygen-induced retinopathy. *Glia* 2008; **56**: 1076-1090
32 **Silva KC**, Rosales MA, Biswas SK, Lopes de Faria JB, Lopes de Faria JM. Diabetic retinal neurodegeneration is associated with mitochondrial oxidative stress and is improved by an angiotensin receptor blocker in a model combining hypertension and diabetes. *Diabetes* 2009; **58**: 1382-1390
33 **Barnstable CJ**, Tombran-Tink J. Neuroprotective and antiangiogenic actions of PEDF in the eye: molecular targets and therapeutic potential. *Prog Retin Eye Res* 2004; **23**: 561-577
34 **Takita H**, Yoneya S, Gehlbach PL, Duh EJ, Wei LL, Mori K. Retinal neuroprotection against ischemic injury mediated by intraocular gene transfer of pigment epithelium-derived factor. *Invest Ophthalmol Vis Sci* 2003; **44**: 4497-4504
35 **Imai D**, Yoneya S, Gehlbach PL, Wei LL, Mori K. Intraocular gene transfer of pigment epithelium-derived factor rescues photoreceptors from light-induced cell death. *J Cell Physiol* 2005; **202**: 570-578
36 **Bilak MM**, Corse AM, Bilak SR, Lehar M, Tombran-Tink J, Kuncl RW. Pigment epithelium-derived factor (PEDF) protects motor neurons from chronic glutamate-mediated neurodegeneration. *J Neuropathol Exp Neurol* 1999; **58**: 719-728
37 **Dawson DW**, Volpert OV, Gillis P, Crawford SE, Xu H, Benedict W, Bouck NP. Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science* 1999; **285**: 245-248
38 **Aymerich MS**, Alberdi EM, Martínez A, Becerra SP. Evidence for pigment epithelium-derived factor receptors in the neural retina. *Invest Ophthalmol Vis Sci* 2001; **42**: 3287-3293
39 **Notari L**, Baladron V, Aroca-Aguilar JD, Balko N, Heredia R, Meyer C, Notario PM, Saravanamuthu S, Nueda ML, Sanchez-Sanchez F, Escribano J, Laborda J, Becerra SP. Identification of a lipase-linked cell membrane receptor for pigment epithelium-derived factor. *J Biol Chem* 2006; **281**: 38022-38037
40 **Filleur S**, Nelius T, de Riese W, Kennedy RC. Characterization of PEDF: a multi-functional serpin family protein. *J Cell Biochem* 2009; **106**: 769-775
41 **Chader GJ**. PEDF: Raising both hopes and questions in controlling angiogenesis. *Proc Natl Acad Sci USA* 2001; **98**: 2122-2124
42 **Tombran-Tink J**, Lara N, Apricio SE, Potluri P, Gee S, Ma JX, Chader G, Barnstable CJ. Retinoic acid and dexamethasone regulate the expression of PEDF in retinal and endothelial cells. *Exp Eye Res* 2004; **78**: 945-955
43 **Cervia D**, Casini G, Bagnoli P. Physiology and pathology of somatostatin in the mammalian retina: a current view. *Mol Cell Endocrinol* 2008; **286**: 112-122
44 **Simó R**, Lecube A, Sararols L, García-Arumí J, Segura RM, Casamitjana R, Hernández C. Deficit of somatostatin-like immunoreactivity in the vitreous fluid of diabetic patients: possible role in the development of proliferative diabetic retinopathy. *Diabetes Care* 2002; **25**: 2282-2286
45 **Hernández C**, Carrasco E, Casamitjana R, Deulofeu R, García-Arumí J, Simó R. Somatostatin molecular variants in the vitreous fluid: a comparative study between diabetic patients with proliferative diabetic retinopathy and nondiabetic control subjects. *Diabetes Care* 2005; **28**: 1941-1947
46 **Lambooij AC**, Kuijpers RW, van Lichtenauer-Kaligis EG, Kliffen M, Baarsma GS, van Hagen PM, Mooy CM. Somatostatin receptor 2A expression in choroidal neovascularization secondary to age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2000; **41**: 2329-2335
47 **Klisovic DD**, O'Dorisio MS, Katz SE, Sall JW, Balster D, O'Dorisio TM, Craig E, Lubow M. Somatostatin receptor gene expression in human ocular tissues: RT-PCR and immunohistochemical study. *Invest Ophthalmol Vis Sci* 2001; **42**: 2193-2201
48 **Davis MI**, Wilson SH, Grant MB. The therapeutic problem of proliferative diabetic retinopathy: targeting somatostatin receptors. *Horm Metab Res* 2001; **33**: 295-299
49 **Simó R**, Carrasco E, Fonollosa A, García-Arumí J, Casamitjana R, Hernández C. Deficit of somatostatin in the vitreous fluid of patients with diabetic macular edema. *Diabetes Care* 2007; **30**: 725-727
50 **Hernández C**, Simó R. Strategies for blocking angiogenesis in diabetic retinopathy by intravitreal therapy. From basic science to clinical practice. *Exp Opin Investig Drug* 2007; **16**: 1209-1226
51 **Marti HH**. Erythropoietin and the hypoxic brain. *J Exp Biol* 2004; **207**: 3233-3242
52 **Hernández C**, Fonollosa A, García-Ramírez M, Higuera M, Catalán R, Miralles A, García-Arumí J, Simó R. Erythropoietin is expressed in the human retina and it is highly elevated in the vitreous fluid of patients with diabetic macular edema. *Diabetes Care* 2006; **29**: 2028-2033
53 **García-Ramírez M**, Hernández C, Simó R. Expression of erythropoietin and its receptor in the human retina: a comparative study of diabetic and nondiabetic subjects. *Diabetes Care* 2008; **31**: 1189-1194
54 **Jelkmann W**. Effects of erythropoietin on brain function. *Curr Pharm Biotechnol* 2005; **6**: 65-79
55 **Becerra SP**, Amaral J. Erythropoietin--an endogenous retinal survival factor. *N Engl J Med* 2002; **347**: 1968-1970
56 **Katsura Y**, Okano T, Matsuno K, Osako M, Kure M, Watanabe T, Iwaki Y, Noritake M, Kosano H, Nishigori H, Matsuoka T. Erythropoietin is highly elevated in vitreous fluid of patients with proliferative diabetic retinopathy. *Diabetes Care* 2005; **28**: 2252-2254
57 **Watanabe D**, Suzuma K, Matsui S, Kurimoto M, Kiryu J, Kita M, Suzuma I, Ohashi H, Ojima T, Murakami T, Kobayashi T, Masuda S, Nagao M, Yoshimura N, Takagi H. Erythropoietin as a retinal angiogenic factor in proliferative diabetic retinopathy. *N Engl J Med* 2005; **353**: 782-792
58 **Inomata Y**, Hirata A, Takahashi E, Kawaji T, Fukushima M, Tanihara H. Elevated erythropoietin in vitreous with ischemic retinal diseases. *Neuroreport* 2004; **15**: 877-879
59 **Jaquet K**, Krause K, Tawakol-Khodai M, Geidel S, Kuck KH. Erythropoietin and VEGF exhibit equal angiogenic potential. *Microvasc Res* 2002; **64**: 326-333
60 **Garcí-Arumí J**, Fonollosa A, Macià C, Hernandez C, Martinez-Castillo V, Boixadera A, Zapata MA, Simo R. Vitreous levels of erythropoietin in patients with macular oedema secondary to retinal vein occlusions: a comparative study with diabetic macular oedema. *Eye* (Lond) 2009; **23**: 1066-1071
61 **Chen J**, Connor KM, Aderman CM, Smith LE. Erythropoietin deficiency decreases vascular stability in mice. *J Clin Invest* 2008; **118**: 526-533
62 **Zhang J**, Wu Y, Jin Y, Ji F, Sinclair SH, Luo Y, Xu G, Lu L, Dai W, Yanoff M, Li W, Xu GT. Intravitreal injection of erythropoietin protects both retinal vascular and neuronal cells in early diabetes. *Invest Ophthalmol Vis Sci* 2008; **49**: 732-742
63 **Grant MB**, Boulton ME, Ljubimov AV. Erythropoietin: when liability becomes asset in neurovascular repair. *J Clin Invest* 2008; **118**: 467-470

S- Editor Zhang HN **L- Editor** Roemmele A **E- Editor** Liu N

World Journal of Diabetes

Online Submissions: http://www.wjgnet.com/1948-9358office
wjd@wjgnet.com
www.wjgnet.com

World J Diabetes 2010 May 15; 1(2): I
ISSN 1948-9358 (online)

Acknowledgments to reviewers of *World Journal of Diabetes*

Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of *World Journal of Diabetes*. The editors and authors of the articles submitted to the journal are grateful to the following reviewers for evaluating the articles (including those published in this issue and those rejected for this issue) during the last editing time period.

Ernest Akingunola Adeghate, PhD, Professor, Department of Anatomy, Faculty of Medicine & Health Sciences, UAE University, PO Box 17666, Al Ain, United Arab Emirates

Min Du, PhD, Associate Professor, Department of Animal Science, University of Wyoming, Laramie, WY 82071, United States

Renu A Kowluru, PhD, Professor of Ophthalmology, Anatomy/Cell Biology, and Endocrinology, K404, Kresge Eye Institute, Wayne State University, 4717 St Antoine, Detroit, MI 48201, United States

Richard E Katholi, MD, Prairie Cardiovascular Consultants, Clinical Professor of Pharmacology and Medicine, Southern Illinois University School of Medicine, Springfield, IL 62794-9420, United States

Gregory I Liou, PhD, Professor, Department of Ophthalmology, Medical College of Georgia, Augusta, GA 30912-3400, United States

Edwin Mariman, PhD, Professor, Department of Human Biology, Maastricht University, 6200 MD Maastricht, The Netherlands

Moshira Abdel Hakim Rateb, MD, Assistant Professor of Human Physiology, Faculty of Medicine (Kasr Al Aini.), Cairo University, Manial El Roda, Cairo, Egypt

World Journal of Diabetes

Online Submissions: http://www.wjgnet.com/1948-9358office
wjd@wjgnet.com
www.wjgnet.com

World J Diabetes 2010 May 15; 1(2): I
ISSN 1948-9358 (online)

Meetings

Events Calendar 2010

January 25-29
Waikoloa, HI, United States
Selected Topics in Internal Medicine

January 28-30
Hong Kong, China
The 1st International Congress on Abdominal Obesity

March 09-12
Brussels, Belgium
30th International Symposium on Intensive Care and Emergency Medicine

March 23-26
Cairo, Egypt
14th Pan Arab Conference on Diabetes PACD14

May 01-05
New Orleans, LA, United States
Digestive Disease Week Annual Meeting

June 09-12
Singapore, Singapore
13th International Conference on Emergency Medicine

June 25-29
Orlando, FL, United States
70th ADA Diabetes Scientific Sessions

September 12-15
Boston, MA, United States
ICAAC: Interscience Conference on Antimicrobial Agents and Chemotherapy Annual Meeting

World Journal of Diabetes

Online Submissions: http://www.wjgnet.com/1948-9358office
wjd@wjgnet.com
www.wjgnet.com

World J Diabetes 2010 May 15; 1(2): I-V
ISSN 1948-9358 (online)

Instructions to authors

GENERAL INFORMATION

World Journal of Diabetes (*World J Diabetes*, *WJD*, online ISSN 1948-9358, DOI: 10.4239), is a bimonthly, open-access (OA), peer-reviewed journal supported by an editorial board of 323 experts in diabetes mellitus research from 38 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results.

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJD* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJD* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJD* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

The major task of *WJD* is to report rapidly the most recent results in basic and clinical research on diabetes including: metabolic syndrome, functions of α, β, δ and PP cells of the pancreatic islets, effect of insulin and insulin resistance, pancreatic islet transplantation, adipose cells and obesity, clinical trials, clinical diagnosis and treatment, rehabilitation, nursing and prevention. This covers epidemiology, etiology, immunology, pathology, genetics, genomics, proteomics, pharmacology, pharmacokinetics, pharmacogenetics, diagnosis and therapeutics. Reports on new techniques for treating diabetes are also welcome.

The columns in the issues of *WJD* will include: (1) Editorial: To introduce and comment on major advances and developments in the field; (2) Frontier: To review representative achievements, comment on the state of current research, and propose directions for future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Basic Research: To provide guidelines for basic research; (6) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (7) Review: To review systemically progress and unresolved problems in the field, comment on the state of current research, and make suggestions for future work; (8) Original Article: To report innovative and original findings in diabetes; (9) Brief Article: To briefly report the novel and innovative findings in diabetes research; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: To discuss and make reply to the contributions published in *WJD*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of diabetes mellitus; and (13) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on basic research and clinical practice in diabetes mellitus.

CSSN
ISSN 1948-9358 (online)

Published by
Beijing Baishideng BioMed Scientific Co., Ltd.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results,

Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Beijing Baishideng BioMed Scientific Co., Ltd, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the International Committee of Medical Journal Editors to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www. clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/1948-9358office. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1948-9358/g_info_20100107165233.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjd@wjgnet.com, or by telephone: +86-10-59080038. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by International Committee of Medical Journal Editors, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g., Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g., Telephone: +86-10-59080039 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJD*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no more than 256 words) and structured abstracts (no more than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no more than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following

sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no more than 140 words); RESULTS (no more than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles, rapid communication and case reports, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/1948-9358/g_info_20100107165233.htm.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc.* It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc*. Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1948-9358/g_info_20100107145507.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.

Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc*.

Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc*.

Biology: *H. pylori*, *E coli*, *etc*.

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies of *WJD*. The revised version including manuscript and high-resolution image figures (if any) should be copied on a floppy or compact disk. The author should send the revised manuscript, along with printed high-resolution color or black and white photos, copyright transfer letter, and responses to the reviewers by courier (such as EMS/DHL).

Editorial Office
World Journal of Diabetes
Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjd@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-59080038
Fax: +86-10-85381893

Language evaluation
The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form
Please download a Copyright assignment form from http://www.wjgnet.com/1948-9358/g_info_20100107144846.htm.

Responses to reviewers
Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/1948-9358/g_info_20100107170340.htm.

Proof of financial support
For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Links to documents related to the manuscript
WJD will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Science news releases
Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee
Authors of accepted articles must pay a publication fee.
EDITORIAL, TOPIC HIGHLIGHTS, BOOK REVIEWS and LETTERS TO THE EDITOR are published free of charge.