ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2017 March 15; 8(3): 89-119

Published by Baishideng Publishing Group Inc

WJD World Journal of Diabetes

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

Editorial Board

2016-2019

The *World Journal of Diabetes* Editorial Board now consists of 676 members, representing a team of worldwide experts in diabetes mellitus. They are from 56 countries, including Argentina (1), Australia (26), Austria (9), Belgium (5), Brazil (11), Canada (24), Chile (3), China (39), Cuba (1), Czech Republic (2), Denmark (12), Egypt (3), Finland (5), France (11), Germany (26), Greece (16), Hungary (4), Iceland (1), India (24), Iran (6), Iraq (2), Ireland (4), Israel (9), Italy (54), Japan (30), Jordan (1), Kuwait (3), Lebanon (1), Malaysia (1), Malta (1), Mexico (4), Netherlands (7), New Zealand (3), Nigeria (2), Norway (2), Oman (3), Pakistan (2), Poland (8), Portugal (1), Qatar (1), Romania (2), Singapore (4), Slovakia (1), South Africa (1), South Korea (15), Spain (25), Sweden (6), Switzerland (3), Thailand (4), Tunisia (1), Turkey (13), United Arab Emirates (3), United Kingdom (28), United States (199), Venezuela (2), and Yemen (1).

Subrata Chakrabarti, *London*
David ZI Cherney, *Toronto*
Mervyn Deitel, *Toronto*
Jean-Pierre Despres, *Québec*
David J Hill, *Ontario*
Tian-Ru Jin, *Toronto*
Arulmozhi D Kandasamy, *Alberta*
Jennifer L Kuk, *Toronto*
Ismail Laher, *Vancouver*
Zhong-Cheng Luo, *Montreal*
Roger S McIntyre, *Toronto*
David Meyre, *Hamilton*
JF Ndisang, *Saskatoon*
Raj S Padwal, *Alberta*
Ciriaco A Piccirillo, *Montreal*
AM James Shapiro, *Edmonton*
Guang Sun, St. *John's*
Valerie Taylor, *Ontario*
Cory Toth, *Calgary*
André Tremblay, *Montréal*
VVincent C Woo, *Manitoba*
James R Wright, *Alberta*
Xi-Long Zheng, *Calgary*

Chile

Sebastian S Martin, *Valparaiso*
Armando Rojas Rubio, *Talca*
Luis Sobrevia, *Santiago*

China

Jie Chen, *Nanjing*
Bernard Man Yung Cheung, *Hong Kong*
William CS Cho, *Hong Kong*
Tian-Pei Hong, *Beijing*
Qin Huang, *Shanghai*
Po-Sing Leung, *Hong Kong*
Chao Liu, *Nanjing*
Jian-Kang Liu, *Xi'an*
Lie-Gang Liu, *Wuhan*
Ronald CW Ma, *Hong Kong*
Zengchang Pang, *Qingdao*
Jin-Sheng Qi, *Shijiazhuang*
Jin-Xiong She, *Shijiazhuang*
Wing Y So, *Hong Kong*
Cheuk C Szeto, *Hong Kong*
Kathryn CB Tan, *Hong Kong*
Cong-Yi Wang, *Wuhan*
Yu Wang, *Hong Kong*
Guang-Da Xiang, *Wuhan*
Bao-Feng Yang, *Harbin*
Shu-Yu Yang, *Xiamen*
Xi-Lin Yang, *Hong Kong*
Zai-Qing Yang, *Wuhan*
Shan-Dong Ye, *Hefei*
Shi-Sheng Zhou, *Dalian*
Zhi-Guang Zhou, *Changsha*

Cuba

Luis Sarmiento-Pérez, *Havana*

Czech Republic

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

Denmark

Charlotte Brons, *Gentofte*
Flemming Dela, *Copenhagen N*
Kristine Faerch, *Gentofte*
Louise G Grunnet, *Gentofte*
R Scott Heller, *Gentofte*
Filip K Knop, *Hellerup*
Helle Markholst, *Måløv*
Ole H Mortensen, *Copenhagen N*
Oluf Pedersen, *Copenhagen K*
Esben T Vestergaard, *Aarhus N*
Milan Zdravkovic, *Soborg*

Egypt

Mamdouh MA Hssan, *Dokki*
Moshira AH Rateb, *Cairo*
Mona F Schaalan, *Cairo*

Finland

Siamak Bidel, *Helsinki*
Gang Hu, *Helsinki*
Thomas Kietzmann, *Oulu*
Qing Qiao, *Espoo*
Karoliina Wehkalampi, *Helsinki*

France

Jean C Ansquer, *Dijon*
Bertrand Cariou, *Nantes*
Sylvie Dejager, *Rueil-Malmaison*
Naim A Khan, *Dijon*
Jean-Philippe Lavigne, *Nimes*
Michel Marre, *Paris*
Marie-Claude Morice, *Massy*
Riccardo Perfetti, *Paris*
Gérard Said, *Paris*
Didier Vieau, *Villeneuve*
Sophie Visvikis-Siest, *Nancy*

Germany

Christa Buechler, *Regensburg*
Roland Büttner, *Heidelberg*
Michael Froehner, *Dresden*
Ioanna Gouni-Berthold, *Cologne*
Hammes Hans-Peter, *Mannheim*
Nadja Herbach, *Munich*
Nadj Herbach, *Munchen*
Andrea Icks, *Düsseldorf*
Thomas Jax, *Neuss*
Michael Kluge, *Munich*
Florian Lang, *Tuebingen*
Matthias Laudes, *Koln*
Ralf Lobmann, *Stuttgart*
Rafael T Mikolajczyk, *Bremen*
Andreas S Mueller, *Halle*
Karsten Müssig, *Tübingen*
Nahid Parvizi, *Mariensee*
Thomas P Reinehr, *Datteln*
Michael Ristow, *Jena*
Sven Schinner, *Duesseldorf*
Peter EH Schwarz, *Dresden*
Ovidiu A Stirban, *Oeynhausen*
Diego J Walther, *Berlin*
Silvia A Wein, *Kiel*
Christian Wrede, *Berlin*
Dan Ziegler, *Düsseldorf*

Greece

George P Chrousos, *Athens*
Moses S Elisaf, *Ioannina*
Panagiotis Georgoulias, *Larissa*
Nikolaos Kadoglou, *Thessaloniki*
Gerasimos E Krassas, *Krini*
Spilios Manolakopoulos, *Athens*
Peppa Melpomeni, *HalDari*
Nikolaos Papanas, *Alexandroupolis*
Dimitrios Papazoglou, *Alexandroupolis*
Sokratis Pastromas, *Athens*
Christina Piperi, *Goudi*
Nicholas K Tentolouris, *Athens*
Konstantinos A Toulis, *Salonika*
Apostolos Tsapas, *Thessaloniki*
Konstantinos Tziomalos, *Thessaloniki*
Elias Zintzaras, *Larissa*

Hungary

Mária Bagyánszki, *Szeged*
Gyorgy Jermendy, *Budapest*
Karoly Racz, *Budapest*
Gyula Soltesz, *Pécs*

Iceland

Saher Hamed, *Haifa*

India

Sarika Arora, *New Delhi*
Pitchai Balakumar, *Sivakasi*
Muthuswamy Balasubramanyam, *Chennai*
Anuradha Carani Venkatraman, *Nagar*
Subhabrata Chakrabarti, *Hyderabad*
Abhay S Chakraborti, *Kolkata*
Tapan K Chaudhuri, *New Delhi*
Kanwaljit Chopra, *Chandigarh*
Malabika Datta, *Delhi*
Debidas Ghosh, *West Bengal*
Ravinder Goswami, *New Delhi*
Jothydev Kesavadev, *Kerala*
KVS H Kumar, *Lucknow*

Anoop Misra, *New Delhi*
Analava Mitra, *Kharagpur*
Viswanathan Mohan, *Chennai*
Pallavi Panchu, *Bangalore*
Deepak N Patel, *Mumbai*
Usharani Pingali, *Hyderabad*
Ambady Ramachandran, *Chennai*
Vadde Ramakrishna, *Kadapa*
Rajat Sandhir, *Chandigarh*
Manju Sharma, *New Delhi*
Suman B Sharma, *Delhi*

Iran

Mohammad K Arababadi, *Rafsanjan*
Leila Azadbakht, *Isfahan*
Hamid Baradaran, *Tehran*
Behrooz Broumand, *Tehran*
Majid Ghayour-Mobarhan, *Mashhad*
Mohsen Janghorbani, *Isfahan*

Iraq

Saad AR Hussain, *Baghdad*
Abbas A Mansour, *Basrah*

Ireland

Amar Agha, *Dublin*
Michael Aviram, *Haifa*
Raymond E Farah, *Safed*
Mark P Hehir, *Dublin*

Israel

Gal Dubnov-Raz, *Hashomer*
Shimon Efrat, *Tel Aviv*
Oren Froy, *Rehovot*
Farid M Nakhoul, *Lower Galilee*
Orit Pinhas-Hamiel, *Ramat-Gan*
Eleazar Shafrir, *Jerusalem*
Gerald H Tomkin, *Dublin*
Haim Werner, *Tel Aviv*
Marina S Zimlichman, *Holon*

Italy

Luigi A Angrisani, *Napoli*
Roberto Baldelli, *Rome*
Giuseppe Barbaro, *Rome*
Alessandro Bartolomucci, *Parma*
Giuseppina Basta, *Pisa*
Simona Bertoli, *Milano*
Federico Bilotta, *Rome*
Fabio Broglio, *Torino*
Riccardo Calafiore, *Perugia*
Sergio Coccheri, *Bologna*
Massimo Collino, *Torino*
Marco A Comaschi, *Genoa*
Renzo Cordera, *Genova*
Francesco Dotta, *Siena*
Fiorucci Fiorucci, *Perugia*
Maurizio Galderisi, *Naples*
Amalia Gastaldelli, *Pisa*
Ezio Ghigo, *Turin*
Carla Giordano, *Palermo*
Paolo Gisondi, *Verona*
Riccarda Granata, *Turin*
Giorgio Iervasi, *Pisa*
Claudia Kusmic, *Pisa*
Francesco Landi, *Rome*
Monica R Loizzo, *Cosenza*
Paolo Magni, *Milan*
Mariano Malaguarnera, *Catania*
Melania Manco, *Rome*
Giulio M Marchesini, *Bologna*
Piero Marchetti, *Pisa*
Massimo Massi-Benedetti, *Perugia*
Moschetta Moschetta, *Bari*
Antonio E Nicolucci, *Milano*
Lucia Pacifico, *Rome*
Stefano Palomba, *Reggio Emilia*
Giampaolo Papi, *Carpi*
Renato Pasquali, *Bologna*
Piermarco M Piatti, *Milano*
Dario Pitocco, *Rome*
Antonio E Pontiroli, *Milano*
Manfredi Rizzo, *Palermo*
Carmelo L Rosa, *Catania*
Raffaella Rosso, *Genoa*
Giuseppe Schillaci, *Perugia*
Leonardo A Sechi, *Sassari*
Imad Sheiban, *Verona*
Cesare R Sirtori, *Milano*
Giovanni Tarantino, *Naples*
Giovanni Targher, *Verona*
Francesco G Tieh, *Chieti*
Donadon Valter, *Pordenone*
Alberto Verrotti, *Chieti*
Andrea Viggiano, *Napoli*
Gian V Zuccotti, *Milan*

Japan

Masato Asahina, *Chiba*
Takuya Awata, *Tochigi*
Yuichiro Eguchi, *Saga*
Goji Hasegawa, *Kyoto*
Satoshi Inoue, *Tokyo*
Eiji Ishimura, *Osaka*
Masayuki Iwano, *Nara*
Takashi Kadowaki, *Tokyo*
Eisuke Kagawa, *Hiroshima*
Masahito Katahira, *Nagoya*
Eiji N Kawasaki, *Nagasaki*
Noriyuki Koibuchi, *Gunma*
Kazuhiko Kotani, *Tochigi*
Daisuke Koya, *Ishikawa*
Norikazu Maeda, *Osaka*
Takayuki Masaki, *Oita*
Yuji Matsuzawa, *Osaka*
Kazuaki Nishio, *Tokyo*
Kenji Okumura, *Nagoya*
Motoaki Saito, *Yonago*
Toshiyasu Sasaoka, *Toyama*
Michio Shimabukuro, *Okinawa*
Kohzo Takebayashi, *Saitama*
Hiroyuki Tamemoto, *Abiko*
Takashi Togo, *Yokohama*
Jun Udagawa, *Izumo*
Yoshinari Uehara, *Fukuoka*
Takuya Watanabe, *Tokyo*
Toshihiko Yada, *Tochigi*
Tohru Yorifuji, *Kyoto*

Jordan

Yousef S Khader, *Irbid*

Kuwait

Kamal AAS Al-Shoumer, *Surra*
Ibrahim F Benter, *Safat*
Abu S Mustafa, *Safat*

Lebanon

Ramzi F Sabra, *Beirut*

Malaysia

Mafauzy Mohamed, *Kota Bharu*

Malta

Charles Savona-Ventura, *Msida*

Mexico

Manuel Gonzalez-Ortiz, *Guadalajara*
Fernando Guerrero-Romero, *Dgo*
Jesus A Olivares-Reyes, *Mexico*
Rocío Salceda, *Mexico*

Netherlands

Sander Kersten, *Wageningen*
Nanne Kleefstra, *Zwolle*
Edwin CM Mariman, *Maastricht*
Frans Pouwer, *Tilburg*
Han Roelofsen, *Groningen*
Suat Simsek, *Alkmaar*
Marcel T Twickler, *Halsterseweg*

New Zealand

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

Nigeria

Adejuwon A Adeneye, *Ikeja*
Anthonia O Ogbera, *Ikeja*

Norway

Akhtar Hussain, *Oslo*
Odd E Johansen, *Hovik*

Oman

Jumana S Saleh, *Muscat*
Mohammed A Shafaee, *Muscat*
Radha Shenoy, *Muscat*

Pakistan

Shahid Hameed, *Islamabad*
Jamil A Malik, *Islamabad*

Poland

Marcin Baranowski, *Bialystok*
Jerzy Beltowski, *Lublin*
Alicia H Dydejczyk, *Krakow*
Maciej Owecki, *Poznań*
Ewa Pankowska, *Warsaw*
Agnieszka Piwowar, *Wroclaw*
Dorota A Zieba, *Krakow*

Portugal

Graca M Pereira, *Braga*

Qatar

Hong Ding, *Doha*

Romania

Elena Ganea, *Bucharest*
Adriana Georgescu, *Bucharest*

Singapore

Thameem T Dheen, *Singapore*
Yung-Seng Lee, *Singapore*
Daniel PK Ng, *Singapore*
Rob M van Dam, *Singapore*

Slovakia

Katarína Šebeková, *Bratislava*

South Africa

Md S Islam, *Durban*

South Korea

Hueng-Sik Choi, *Gwangju*
Kyung M Choi, *Seoul*
Won M Hwang, *Seoul*
Eui-Bae Jeung, *Cheongju*
Ju-Hee Kang, *Incheon*
Sin-Gon Kim, *Seongbuk-Gu*
Sung-Jin Kim, *Seoul*
Young-Gyu Ko, *Seoul*
Kang-Beom Kwon, *Chonbuk*
Sangyeoup Lee, *Yangsan*
Myung Gull Lee, *Gyeonggi-Do*
Soo Lim, *Seongnam*
Byung-Hyun Park, *Jeonbuk*
Seungjoon Park, *Seoul*
Jeesuk Yu, *Chungnam*

Spain

Vivencio Barrios, *Madrid*
M. Luisa Bonet, *Palma de Mallorca*
Justo P Castano, *Cordoba*
Manuel A Diosdado, *Cádiz*
Javier Espino, *Badajoz*
Ricardo V García-Mayor, *Vigo*
José M Gómez-Sáez, *Barcelona*
Oreste Gualillo, *Santiago de Compostela*
Emilio Herrera, *Madrid*
Amelia Marti, *Pamplona*
Navarra JA Martínez, *Pamplona*
Maria L Martinez-Chantar, *Derio*
Merce Miranda, *Tarragona*
Alberto Ortiz, *Madrid*
Maria J Ramirez, *Pamplona*
Eugenia Resmini, *Barcelona*
Pedro Romero-Aroca, *Reus*
Jordi Salas-Salvado, *Reus*
Gines M Salido, *Caceres*
Victor Sanchez-Margalet, *Seville*
Helmut Schroder, *Barcelona*
Carmen Segundo, *Cadiz*
Rafael Simo, *Barcelona*
Manuel Vazquez-Carrera, *Barcelona*

Sweden

Joanna Hlebowicz, *Malmö*
Peter Lindgren, *Stockholm*
Kaj S Stenlof, *Göteborg*
Ann-Britt Wirehn, *Linköping*
Wei-Li Xu, *Stockholm*
Shao-Nian Yang, *Stockholm*

Switzerland

Kaspar Berneis, *Zurich*
Kim-Anne Le, *Lausanne*
Christian Toso, *Geneva*

Thailand

Narattaphol Charoenphandhu, *Bangkok*
Arthorn Riewpaiboon, *Bangkok*
Rawee Teanpaisan, *Hat-Yai*
Viroj Wiwanitkit, *Bangkok*

Tunisia

Khaled Hamden, *Sfax*

Turkey

Ugur Cavlak, *Denizli*
Teoman Dogru, *Etlik*
Ersin Fadillioglu, *Ankara*
Abdurrahman F Fidan, *Afyonkarahisar*
Muammer Karadeniz, *Bornova-Izmir*
Cevde Kaya, *Istanbul*
Fahrettin Kelestimur, *Kayseri*
Altan Onat, *Istanbul*
Semir Ozdemir, *Antalya*
Mustafa Sahin, *Ankara*
Ilker Tasci, *Ankara*
Belma Turan, *Ankara*
Serap Yalin, *Mersin*

United Arab Emirates

Ernest Akingunola Adeghate, *Al Ain*
Mukesh M Agarwal, *Al Ain*
Samir M Awadallah, *Sharjah*

United Kingdom

Nisreen Alwan, *Leeds*
Bing Chen, *Liverpool*
Fay Crawford, *Edinburgh*
Timothy M Curtis, *Belfast*
Umesh Dashora, *Edinburgh*
Gareth W Davison, *Belfast*
Peter Flatt, *Coleraine*
Kathleen M Gillespie, *Bristol*
Peter J Grant, *Leeds*
Lorna W Harries, *Exeter*
Nigel Hoggard, *Aberdeen*
Nigel Irwin, *Coleraine*
Pappachan Joseph, *London*
Andreas F Kolb, *Aberdeen*
Moffat J Nyirenda, *Edinburgh*
Jeetesh V Patel, *Birmingham*
Snorri B Rafnsson, *Edinburgh*
Thozhukat Sathyapalan, *Yorkshire*
Latika Sibal, *Newcastle*
Rajagopalan Sriraman, *Lincoln*
Ramasamyiyer Swaminathan, *London*
Abd A Tahrani, *Birmingham*
Neil G Thomas, *Birmingham*
Cecil Thompson, *London*
Paul H Whiting, *Leicester*

United States

Varun Agrawal, *Springfield*

Pascale Alard, *Louisville*
Omar Ali, *Milwaukee*
Mohamed AS Al-Shabrawey, *Augusta*
Judith Aponte, *New York*
Balamurugan N Appakalai, *Louisville*
Hwyda A Arafat, *Philadelphia*
Carl V Asche, *Salt Lake City*
Sanford A Asher, *Pittsburgh*
Anthony Atala, *Winston-Salem*
Sami T Azar, *New York*
George L Bakris, *Chicago*
Alistair J Barber, *Hershey*
Daniel C Batlle, *Chicago*
David SH Bell, *Birmingham*
Rita Bortell, *Worcester*
Sebastien G Bouret, *Los Angeles*
Donald W Bowden, *Winston-Salem*
David L Brown, *Stony Brook*
Jack D Caldwell, *Erie*
Anna C Calkin, *Los Angeles*
Roberto A Calle, *Groton*
Keith R Campbell, *Pullman*
Carlos Campos, *New Braunfels*
Heping Cao, *New Orleans*
Krista Casazza, *Birmingham*
Aaron B Caughey, *Portland*
Eileen R Chasens, *Pittsburgh*
Munmun Chattopadhyay, *Ann Arbor*
Xiao-Li Chen, *St Paul*
Craig I Coleman, *Hartford*
Robert Conley, *Indianapolis*
Colleen Croniger, *Cleveland*
Doyle M Cummings, *Greenville*
William C Cushman, *Memphis*
Patricia Darbishire, *West Lafayette*
Guillaume Darrasse-Jèze, *New York*
Ravi KM Dasu, *Sacramento*
Michael H Davidson, *Chicago*
Prakash Deedwania, *San Francisco*
Hong-Wen Deng, *Kansas City*
Teresa P DiLorenzo, *Bronx*
Scot Dowd, *Lubbock*
Samuel Durso, *Baltimore*
Krystal Edwards, *Dallas*
Alexander M Efanov, *Indianapolis*
Azza B El-Remessy, *Augusta*
Amy Z Fan, *Atlanta*
Melissa S Faulkner, *Tucson*
George S Ferzli, *Staten Island*
Paolo Fiorina, *Boston*
James E Foley, *East Hanover*
Samuel N Forjuoh, *Temple*
Alessia Fornoni, *Miami*
Trudy Gaillard, *Columbus*
Pietro Galassetti, *Irvine*
Claudia Gragnoli, *Hershey*
Jennifer B Green, *Durham*
Alok K Gupta, *Piscataway*
Gary J Grover, *Piscataway*
Werner Gurr, *New Haven*
Samy L Habib, *San Antonio*
Abdel Hamad, *Baltimore*
Tiffany Hilton, *Pittsford*
Michael F Holick, *Boston*
Zhaoyong Hu, *Houston*
Rachel Hudacko, *Suffern*
Yasuo Ido, *Boston*
Brian K Irons, *Lubbock*
Pamela Itkin-Ansari, *La Jolla*
Hieronim Jakubowski, *Newark*
Hong-Lin Jiang, *Blacksburg*
Ping Jiao, *Providence*
Shengkan Jin, *Piscataway*
Arpita Kalla, *St Louis*
Richard E Katholi, *Springfield*
Melina R Kibbe, *Chicago*
Bhumsoo Kim, *Ann Arbor*
Tomoshige Kino, *Bethesda*
Julienne K Kirk, *Winston-Salem*
Renu A Kowluru, *Detroit*
Lewis H Kuller, *Pittsburgh*
Rajesh Kumar, *Temple*
Blandine Laferrere, *New York*
Cong-Jun Li, *Beltsville*
Ching-Shwun Lin, *San Francisco*
James F List, *Princeton*
Dongmin Liu, *Blacksburg*
Zhen-Qi Liu, *Charlottesville*
Maria F Lopes-Virella, *Charleston*
Cai Lu, *Louisville*
George W Lyerly Jr, *Conway*
Jian-Xing Ma, *Oklahoma City*
Xin-Laing Ma, *Philadelphia*
Rong Ma, *Fort Worth*
David Maggs, *San Diego*
Kenneth Maiese, *Newark*
Kevin C Maki, *Glen Ellyn*
Sridhar Mani, *Bronx*
Suresh Mathews, *Auburn*
Lauraar R McCabe, *East Lansing*
Sarah Messiah, *Miami*
Thomas O Metz, *Richland*
Shannon Miller, *Orlando*
Murielle Mimeault, *Omaha*
Raghu G Mirmira, *Indianapolis*
Prasun J Mishra, *Bethesda*
Reema Mody, *Grayslake*
Arshag D Mooradian, *Jacksonville*
Mohammad-Reza Movahed, *Tucson*
Yingjun J Mu, *Rahway*
Nair G Muraleedharan, *East Lansing*
Manuel F Navedo, *Seattle*
Charles B Nemeroff, *Atlanta*
Joshua J Neumiller, *Spokane*
Steven J Nicholls, *Cleveland*
Hirofumi Noguchi, *Dallas*
Craig S Nunemaker, *Charlottesville*
Patrick J O'Connor, *Minneapolis*
Wei-Hong Pan, *Baton Rouge*
Naushira Pandya, *Fort Lauderdale*
Michael R Peluso, *Corvallis*
Inga Peter, *New York*
Axel Pflueger, *Rochester*
Gretchen A Piatt, *Pittsburgh*
John D Piette, *Ann Arbor*
Leonid Poretsky, *New York*
Parviz M Pour, *Omaha*
Wei Qiu, *Boston*
Teresa Quattrin, *Buffalo*
Cristina Rabadán-Diehl, *Bethesda*
Rajendra S Raghow, *Memphis*
Swapnil N Rajpathak, *Bronx*
Armin Rashidi, *Norfolk*
Mohammed S Razzaque, *Boston*
Beverly AS Reyes, *Philadelphia*
Shuo L Rios, *Los Angeles*
David Rodbard, *Potomac*
Helena W Rodbard, *Rockville*
June H Romeo, *Cleveland*
Raul J Rosenthal, *Florida*
Juan M Saavedra, *Bethesda*
Frank AJL Scheer, *Boston*
Richard E Scranton, *Tiverton*
Vallabh R Shah, *Albuquerque*
Aziz Shaibani, *Houston*
Guo-Ping Shi, *Boston*
Carol A Shively, *Winston-Salem*
Anders AF Sima, *Detroit*
Rajan Singh, Los *Angeles*
Pramil N Singh, *Loma Linda*
Dawn D Smiley, *Atlanta*
Matthew D Solomon, *Stanford*
Rakesh K Srivastava, *Tyler*
Bangyan L Stiles, *Los Angeles*
Erin St Onge, *Apopka*
Yu-Xiang Sun, *Houston*
Salim Surani, *Corpus Christi*
Arthur LM Swislocki, *Martinez*
Ya-Xiong Tao, *Auburn*
John A Tayek, *Torrance*
John G Teeter, *New Haven*
Carlos M Telleria, *Vermillion*
Christophe G Thanos, *Providence*
Ronald G Tilton, *Galveston*
Serena Tonstad, *Loma Linda*
Michael Traub, *Staten Island*
Margrit Urbanek, *Chicago*
Vladimir N Uversky, *Indianapolis*
Gabriel Uwaifo, *Baton Rouge*
Volker Vallon, *San Diego*
Shambhu D Varma, *Baltilmore*
Chengming Wang, *Auburn*
Hong-Jun Wang, *Boston*
Mark E Williams, *Boston*
Guang-Yu Wu, *New Orleans*
Zhong-Jian Xie, *San Francisoco*
Ming-Zhao Xing, *Baltimore*
Hariom Yadav, *Bethesda*
Lijun Yang, *Gainesville*
Ruojing Yang, *Rahway*
Subhashini Yaturu, *Albany*
Joseph Yeboah, *Charlottesville*
Dengping Yin, *Nashville*
Yi-Sang Yoon, *Rochester*
Yi-Hao Yu, *New York*
Kevin CJ Yuen, *Portland*
Ian S Zagon, *Hershey*

Robert YL Zee, *Boston*
Cui-Lin Zhang, Rockville
James X Zhang, Richmond
Sarah X Zhang, Oklahoma City
Guixiang Zhao, *Atlanta*
Yang Zhao, *Carmel*

Ming-Hui Zou, *Oklahoma City*

Venezuela

José F Arévalo, *San Bernardino*

Fuad Lechin, *Caracas*

Yemen

Khaled AA Ahmed, *Ibb*

World Journal of Diabetes

Contents

Monthly Volume 8 Number 3 March 15, 2017

MINIREVIEWS

ORIGINAL ARTICLE

Retrospective Cohort Study

Prospective Study

World Journal of Diabetes
Volume 8 Number 3 March 15, 2017

Contents

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
April 15, 2010

FREQUENCY
Monthly

EDITORIAL OFFICE
Xiu-Xia Song, Director
World Journal of Diabetes
Baishideng Publishing Group Inc
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Inc
8226 Regency Drive,
Pleasanton, CA 94588, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
March 15, 2017

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/ *World J Diabetes* 2017 March 15; 8(3): 89-96

DOI: 10.4239/wjd.v8.i3.89 ISSN 1948-9358 (online)

MINIREVIEWS

Management of critically ill patients with diabetes

Livier Josefina Silva-Perez, Mario Alberto Benitez-Lopez, Joseph Varon, Salim Surani

Livier Josefina Silva-Perez, Mario Alberto Benitez-Lopez, Department of Medicine, Autonomous University of Baja California, School of Medicine, Tijuana, BC 22260, Mexico

Joseph Varon, Department of Medicine, Foundation Surgical Hospital, the University of Texas Health Science Center at Houston, Houston, TX 77030, United States

Salim Surani, Department of Medicine, Texas A and M University, Corpus Christi, TX 78413, United States

Author contributions: Silva-Perez LJ and Benitez-Lopez MA was involved in writing, preparing figures and tables; Silva-Perez LJ and Benitez-Lopez MA contributed equally to this work; Surani S outlined and coordinated the writing of the paper; served as primary consultant for the paper, was the primary reviewer of the paper and added significant contributions to the text; Varon J provided input in writing the paper and made significant contributions to the text.

Conflict-of-interest statement: None of the authors have any conflict of interest to disclose as it regards to this manuscript.

Manuscript source: Invited manuscript

Correspondence to: Salim Surani, MD, MPH, MSHM, FACP, FCCP, FAASM, Department of Medicine, Texas A and M University, Corpus Christi, 1177 West Wheeler Ave, Suite 117, Aransas Pass, TX 78413, United States. srsurani@hotmail.com
Telephone: +1-361-8857722
Fax: +1-361-8507563

Received: August 27, 2016
Peer-review started: August 29, 2016
First decision: November 11, 2016
Revised: November 30, 2016
Accepted: December 27, 2016

Article in press: December 29, 2016
Published online: March 15, 2017

Abstract

Disorders of glucose homeostasis, such as stress-induced hypoglycemia and hyperglycemia, are common complications in patients in the intensive care unit. Patients with preexisting diabetes mellitus (DM) are more susceptible to hyperglycemia, as well as a higher risk from glucose overcorrection, that may results in severe hypoglycemia. In critically ill patients with DM, it is recommended to maintain a blood glucose range between 140-180 mg/dL. In neurological patients and surgical patients, tighter glycemic control (*i.e.*, 110-140 mg/d) is recommended if hypoglycemia can be properly avoided. There is limited evidence that shows that critically ill diabetic patients with a glycosylated hemoglobin levels above 7% may benefit from looser glycemic control, in order to reduce the risk of hypoglycemia and significant glycemic variability.

Key words: Diabetes mellitus; Critical care; Stress hyperglycemia; Hypoglycemia; Glycemic control; Intensive care unit

Core tip: Diabetes mellitus is a common comorbidity found in critically ill patients. Although strict glycemic control in the past was considered a standard therapeutic intervention, newer clinical trials have shown that moderate glycemic control (*i.e.*, glucose levels between 140-180 mg/dL) reduces mortality and morbidity in such patients.

Silva-Perez LJ, Benitez-Lopez MA, Varon J, Surani S. Management of critically ill patients with diabetes. *World J Diabetes* 2017; 8(3): 89-96 Available from: URL: http://www.wjgnet.com/1948-9358/full/v8/i3/89.htm DOI: http://dx.doi.org/10.4239/

wjd.v8.i3.89

INTRODUCTION

Stress-induced hyperglycemia, or diabetes injury as originally described by Claude Bernard in 1877, has become the subject of significant debate, as recent guidelines have called for stricter glucose control in critically ill patients[1,2]. Occurring as a result of catecholamine-induced stress response, this hyperglycemia is a common occurrence in critically ill patients[1]. With the rising population of diabetic and pre-diabetic individuals in the United States, the risk of severe hyperglycemia among critically ill patients is quite high, particularly in patients with undiagnosed diabetes mellitus (DM), who have inadequate glycemic control previous to hospitalization[1,3].

On the other hand, one of the important complications in dealing with stress-induced hyperglycemia is severe hypoglycemia. This significant decrease in blood glucose, however, is not due to some underlying physiological process, but it is often the consequence of inadequate glucose monitoring, and incorrect dosage of hypoglycemic medication, usually insulin. Hypoglycemia in critically ill patients is an important factor that can increase mortality in the intensive care unit (ICU), and is an important complication that needs to be prevented in patients that require glycemic control therapy[4]. Increased glycemic variability may be an issue with inadequate hypoglycemic treatment, which leads to increased oxidative stress and may be more dangerous than persistent hyperglycemia[5].

Appropriate hypoglycemic therapy is required in order to reduce mortality and morbidity of uncontrolled hyperglycemia in critically ill patients[6]. In this article, we review the current state-of-evidence on ideal glycemic goals that should be set for diabetic patients in the ICU.

EPIDEMIOLOGY

In 2014, the United States National Diabetes Statistic Report, documented 21 million individuals suffering from DM, accounting for 6.7% of the total population and approximately 8.1 million undiagnosed DM, which would raise the percentage of American population with diabetes to 9.3%[7]. This report also indicated that the prevalence of diabetes was highest among those older than 65 years of age and above[7]. Patients in this age group, account for up to 45.7% of ICU patients[8]. In addition, approximately 50% of ICU patients, have pre-existing diagnostic criteria for DM[9].

PATHOPHYSIOLOGY

During periods of stress, the body reacts by producing elevated levels of catecholamines[10]. This reaction, is modulated by the suprarenal glands and activated by either the sympathetic nervous system in acute stress and by feedback to the pituitary gland in chronic stress[11,12]. Any period of disease can be considered a period of stress, and therefore, some degree of hyperglycemia is normal during these times, and can be seen as initially protective and part of the adaptive response for survival[13]. However, in acute and severe diseases, the resulting hyperglycemia can be much too high and require glycemic control therapy to manage[1].

Severe hyperglycemia, is a well-documented marker of illness severity, rather than a direct cause of poor outcome[13]. This condition often subsides after the affecting illness (*i.e.*, sepsis) has resolved[1]. In the acute setting, it is believed that the resulting hyperglycemia is due to insufficient insulin secretion that is unable to overcome the hyperglycemic effect of catecholamine.[14] It is also believed that insulin resistance plays a factor in chronic disease with significant amounts of tissue injury[1,14].

Patients with pre-existing DM tend to have a persistent state of hyperglycemia due to insulin resistance (or insulin absence in DM type 1), and hyperglucagonaemia that are the consequences of the disease's natural progression. As a result of these factors, during periods of acute illness, the resulting stress-induced hyperglycemia can be much more severe than in non-diabetic patients, and more likely to require control with hypoglycemic medications and strict glucose monitoring[14]. See Table 1 for factors that lead to hyperglycemia and hypoglycemia in critically ill patients.

STRESS-INDUCED HYPERGLYCEMIA

Stress-induced hyperglycemia (SIH) is a common finding among critically ill patients, particularly among cardiovascular patients, neurocritical patients, and patients undergoing surgical procedures, even in the absence of preexisting DM[14]. In non-diabetic patients, SIH has been arbitrarily defined as a blood glucose level greater than 140 mg/dL or glycosylated hemoglobin (HbA1c) greater than 6.5%[15]. In diabetic patients, SIH is be defined as blood glucose levels greater than 180-220 mg/dL[15]. This clinical condition increases the morbidity and mortality in critically ill patients and leads to poor outcomes and prognosis[15]. Some have advocated that in these patients, it is necessary to maintain a strict glycemic control to directly improve their outcomes[14,15].

Part of the controversy as to the precise level of strict glycemic control started with a clinical study published in 2001, consisting of 1548 patients in a surgical ICU in Belgium[16]. In this study, van den Berghe *et al*[16] reported that intensive insulin therapy, aimed at maintaining blood glucose below 110 mg/dL reduced mortality and morbidity in critically ill patients by 42%. The reduction in mortality was apparent among patients who stayed in the ICU for more than five days[16]. A follow-up study, by the same investigators in 2006, aimed at comparing strict blood glycemic control (blood glucose: 80-110 mg/dL) *vs* a much looser control (blood glucose: 180-215 mg/dL) in this study on 1200 medical

Table 1 Factors leading to hyperglycemia and hypoglycemia in critically ill patients

Hyperglycemia[65]	Hypoglycemia[66]
Release of stress hormones (glucagon, epinephrine, cortisol, and TNF-α)	Severe sepsis
Certain medications (exogenous glucocorticoids, vasopressors, lithium, and β-blockers)	Trauma
Overfeeding	DM
Intravenous dextrose	Prior insulin treatment
Parenteral nutrition	Prior glucocorticoid treatment
Persistent bed rest	Cardiovascular failure
Increased insulin resistance (DM type 2)	Intensive glucose control
Deficient insulin secretion (DM type 1)	

DM: Diabetes mellitus; TNF: Tumor necrosis factor.

ICU patients and found that the strict glucose control group had a mortality reduction rate of 32% in patients who stayed more than three days in the ICU[17]. Of note, in this study, strict glucose control increased mortality in patients with short ICU stays (< 3 d), due to the increased rate of severe hypoglycemia.

A series of additional clinical trials followed these 2 seminal investigations. One of the most quoted in the medical literature was the Normoglycemia in Intensive Care Evaluation-Survival Using Glucose Algorithm Regulation (NICE-SUGAR) multicenter trial, with 6104 ICU patients that compared strict glucose control (81-108 mg/dL) *vs* a more moderate glucose target (< 180 mg/dL)[18]. This study reported that moderate glycemic control lead to a reduction in cardiovascular mortality in critically ill patients.

Glycemic variability and hypoglycemia

As noted above, in diabetic patients, particularly those with persistent hyperglycemia, significantly lowering glucose levels and strict glycemic control may lead to symptomatic and life-threatening hypoglycemia and glycemic variability[19]. Glycemic variability has been defined as acute glycemic fluctuations; with both upwards fluctuations (in hypoglycemic correction) and downward fluctuations (in initial overbearing hypoglycemic treatment) leading to increased oxidative stress (which in turn leads to endothelial dysfunction and vascular damage). It is well documented that glycemic variability is much more dangerous than persistent hyperglycemia in critically ill patients[19,20].

Two retrospective studies found that glycemic variability conferred an increased risk of mortality in critically ill patients[21,22]. The mortality risk increased by 25.7% in critically ill non-diabetic patients[21,22]. Although no current consensus exists on the adequate range of acceptable glycemic variability in critically ill patients, Monnier and associates proposed a range of 40 mg/dL, as this corresponds to the normal variability found in non-diabetic healthy individuals[20].

Hypoglycemia is another dangerous situation in both diabetic and non-diabetic ICU patients. This clinical entity is directly related to cardiovascular mortality as it has been associated with increased QT waves in the electrocardiogram and changes in cardiac cell repolarization[23,24]. A study performed in 2005 reported that diabetic patients hospitalized with acute myocardial infarction, had a 93% increased mortality rate when hypoglycemia was present during their hospitalization[25]. In another study published last year, 2601 patients were evaluated and analyzed ICU mortality when moderate or severe hypoglycemia was present as compared to no hypoglycemia. Patients with severe and moderate hypoglycemia had a 34% and 18% increase, respectively, in ninty days mortality, when compared to patients with no hypoglycemia. Those patients that presented multiple hypoglycemic events had a 44% increase in mortality when compared to patients with no hypoglycemic events[26].

There is significant evidence that hypoglycemia poses significant risk of cardiovascular mortality among diabetic patients in critical care scenarios. Alongside the theoretical benefits of reducing glycemic variability, having a much looser glycemic control in critically ill diabetic patients, may aid in reducing cardiovascular mortality[27]. Further studies are necessary on the subject of glycemic variability, in an effort to find its real-world impact on diabetic patients in and out of critical care.

GUIDELINES RECOMMENDATIONS

The American Diabetes Association recommends starting insulin in patients with persistent hyperglycemia above 180 mg/dL in critically ill patients, and to maintain the glycemic range between 140-180 mg/dL. It also states that stricter glycemic control (110-140 mg/dL) can be appropriate for certain patients, such as patients with acute cardiac ischemia or patients with acute neurological event, as long as significant hypoglycemia can be avoided[28]. They also recommend active prevention of hypoglycemia by having a treatment plan if hypoglycemia were to develop and to change the current therapy if serum glucose levels fall below 70 mg/dL[28]. These recommendations were based on a consensus form American Association of Clinical Endocrinologists, which involved two meta-analyses of several clinical trials, including the NICE-SUGAR study, the largest randomized controlled trial, addressing this issue[28-31].

The American College of Physicians recommends serum glucose levels between 140-200 mg/dL indepen-

dent of diabetic status, and recommends avoiding blood sugar levels below 140 mg/d, due to the associated risks of hypoglycemia and glycemic variability[32]. The Society of Critical Care Medicine (SCCM) recommends maintaining the serum glucose level between 150-180 mg/dL[33].

However, a 2011 study conducted in the ICU among diabetic patients found that patients with uncontrolled diabetes (HbA1c above 7%) had different mortality when hyperglycemia was present when compared to non-diabetic patients or patients with better controlled diabetes (HbA1c below 7%)[34]. Additional newer studies have concluded similarly, that diabetic patients do not share the same mortality with hyperglycemia as non-diabetic patients, and that these diabetic patients may benefit from higher glycemic ranges to reduce the risk of hypoglycemia and glycemic variability[35-37]. Moreover, another study recommended maintaining serum glucose levels between 160-220 mg/dL in patients with HbA1c above 7%, and to maintain serum glucose levels between 140-200 mg/dL in patients with an HbA1c below 7%[19].

It is recommended that glycemic control be maintained with insulin due to the effectiveness, quick action, and few contraindications as it relates to this therapy[28,29]. However, the use of continuing metformin therapy in ICU patients with type 2 diabetes is seeing resurgence among certain patients, as the risk of hypoglycemia is lower; although its use should be cautious among patient with renal insufficiency, which is very common in the ICU[38].

In the following sections, we describe the evidence and recommendations for glycemic control among different patient groups who may be presenting in the ICU. Details are depicted in Table 2.

Patients in the surgical ICU

The Society of Thoracic Surgeons created guidelines in 2009 for glucose management in adult cardiac surgery patients, including diabetics[39]. For preoperative care, maintenance with insulin therapy with a serum glucose goal below 180 mg/dL was recommended. It was also recommended to check HbA1c level pre-operative for proper glycemic management. Intraopertavely, insulin therapy was also recommended for glycemic values above 180 mg/dL, and intravenous insulin infusion was recommended for persistent glycemic levels above 180 mg/dL intra-operatively or postoperatively in the ICU[39]. The recommendation was to keep a goal of 180 mg/dL throughout their stay in the ICU unless they are expected to remain in the critical care unit more than 3 d, or if the patient is ventilator-dependent, or requires therapy with inotropes, intra-aortic balloon pump, left ventricular assist device, anti-dysrhythmic medications, dialysis, or hemofiltration. In aforementioned cases, it is recommended to have the blood glucose levels below 150 mg/dL[39,40].

A recent study in 447 patients, found that a glucose level of 80-110 mg/dL, when compared to 140-180 mg/dL, reduces surgical site infections[41]. However, this study did not focus on over-all patient mortality and had a challenge of small sample size.

Patients with neurological events

A large clinical trial by van den Berghe *et al*[16] in 2001, suggested that strict glucose control (< 110 mg/dL) reduces mortality in critically ill patients[16]. For a period of time, following the findings of this trial, the standard of care was to maintain neurocritically ill patients blood glucose below 110 mg/dL[16]. However, the publication of the NICE-SUGAR study, and a prospective study of intensive insulin therapy in patients with recent neurosurgery, both published in 2009, showed that strict glucose control led to increased mortality mainly secondary to hypoglycemia[18,42].

In 2012, a systematic review and meta-analysis of 16 clinical trials on optimal glycemic control in neurocritical care patients, revealed that strict glycemic control (70-140 mg/dL) had no impact on patient mortality, but did increased the incidence of hypoglycemia[43]. Loose glycemic control (> 200 mg/dL) was shown to increased mortality when compared to a moderate glycemic control (140-180 mg/dL)[43]. The ADA states that blood glucose level of 110-140 mg/dL may be appropriate if significant hypoglycemia can be avoided[28].

Patient with an acute myocardial infarction

In 2008, the American Heart Association released a statement on glucose management in acute coronary syndrome, which recommended a glucose levels between 90-140 mg/dL in ICU patients with acute coronary syndrome[44]. The recommendations were later updated in 2009, suggesting an upper limit of serum glucose to 180 mg/dL[45].

The European Society of Cardiology published their most recent guidelines in 2012 on management of acute myocardial infarction with ST-segment elevation[46]. They recommend loose glycemic control in the acute phase, by maintaining the patient serum glucose below 200 mg/dL, as hypoglycemia was felt to be an important factor which increases the mortality[46]. This conclusion is based on a consensus reached by the National Institute Health and Care Excellence in 2011, that stated that no high quality studies were available to reach an evidence-based conclusion[47].

A 2012 meta-analysis, focusing on type 2 diabetics with acute myocardial infarction, involving 3 studies (for a total of 2113 patients), concluded that stricter glucose control with intensive insulin therapy did not reduced the patient mortality but significantly increased the incidence of hypoglycemia while offering no overall reduction in cardiovascular mortality[48].

Patients with sepsis

In response to the study on glucose control in surgical ICU patients, a study specifically on patients with sepsis, the Surviving Sepsis Campaign recommended a

Table 2 Glycemic control recommendation based on patient condition

Condition	Glucose control recommendation	Studies with patient number	Ref.
Non-diabetic ICU patients	140-180 mg/dL	29 studies with 8432 total patients and 26 studies with 13567 total patients	Wiener *et al*[30] (2008) and Griesdale *et al*[31] (2009), respectively
Diabetic ICU patients	If HbA1c < 7%: 140-180 mg/dL If HbA1c > 7%: > 200 mg/dL	1 retrospective study with 415 total patients	Egi *et al*[34] (2011)
Surgical ICU	If ICU stay is for more than 3 d, ventilator dependent, on dialysis, or with cardiac comorbidities: < 150 mg/dL	1 prospective study with 4864 total patients across 17 yr	Furnary *et al*[40] (2004)
Neurocritical ICU patients	If not: < 180 mg/dL If hypoglycemia can be prevented: 110-140 mg/dL If not: 140-180 mg/dL	16 studies with 1258 total patients	Kramer *et al*[43] (2012)
STEMI ICU patients	< 200 mg/dL	No high quality studies available Consensus by NICE	Nice Guidelines[47] (2011)
Sepsis ICU patients	< 180 mg/dL	1 randomized control trial with 6104 patients	Based of NICE-SUGAR study[17]
Pregnant ICU patients	No consensus	N/A	Van de Velde *et al*[55] (2013)

ICU: Intensive care unit; N/A: Not applicable; HbA1c: Glycosylated hemoglobin; NICE-SUGAR: Normoglycemia in Intensive Care Evaluation-Survival Using Glucose Algorithm Regulation.

stricter range of glycemic control, with an upper goal of 110 mg/dL of serum glucose[17,49,50]. With the advent of the NICE-SUGAR trial in 2009, which also included septic patients, the 2013 update of the Surviving Sepsis Campaign modified its recommendation to a looser goal of 180 mg/dL[51]. Due to increased risk of hypoglycemia and hypoglycemia-related mortality, the Surviving Sepsis Campaign deemed that there was no apparent benefit from strict glucose control[51]. Insulin therapy was recommended to be started after two consecutive blood glucose measurements were above 180 mg/dL and to maintain a blood glucose of less than 180 mg/dL[51].

Pregnant patients

Gestational diabetes accounts for 2% to 9% of all pregnancies[52]. Hyperglycemia is an important factor to consider in all pregnancies, especially among hospitalized patients. During pregnancy, maternal cells have increased insulin resistance, due to elevated levels of human placental lactogen, progesterone, and estrogen[53]. This mild increase in insulin resistance is protective, and allows glucose absorption to be prioritized in the fetus, however in some patients, this mild resistance can be combined with insulin resistance, leading to persistent hyperglycemia[53,54].

It is generally agreed that treatment of gestational diabetes-related hyperglycemia is important in reducing perinatal mortality, as well as reducing hyperglycemia in postpartum mothers and improving overall health[52]. No consensus currently exists on the ideal range of serum glucose levels in critically ill pregnant patients[55]. It is difficult to recommend moderate or loose glycemic control in these patients, as even mild hyperglycemia can lead to adverse outcomes in infants[56]. On the other hand, tight glycemic control may lead to increased risk of hypoglycemia, which is also a factor that increases both maternal and infant mortality. Future clinical trials are necessary to be able to reach a consensus on how glycemic care should be managed in this population.

GLYCEMIC CONTROL THERAPY

While several studies have been performed on glycemic control in non-diabetic patients in the ICU, few of such studies have been performed on diabetic individuals. Table 3 depicts recent studies on this topic. Three of the four studies focused on surgical patients, and recommend a stricter glucose control for infection prevention, and hyperglycemia prevention[57-59]. The fourth study takes into account the risk of hypoglycemia, and recommends looser glycemic control to reduce moderate to severe hypoglycemia and glycemic variability[9]. However, all of these studies fail to take into account that diabetic individuals with persistent hyperglycemia (HbA1c above 7%) who are at higher risk from hypoglycemia-related mortality than hyperglycemia-related mortality[19,34]. A 2016 study on diabetic ICU patients, recommended keeping serum glucose levels below 250 mg/dL in patients with HbA1c above 7% upon admission to the ICU[9]. This study found that this loose glycemic control prevented glycemic variability and reduced the incidence of moderate and severe hypoglycemia[9].

Measurement of glucose should be performed every 2 to 4 h to allow for proper monitoring. If the patient's serum glucose concentration is fluctuating, it may be necessary to measure glucose every 30 or 60 min[60]. Currently, technology for continuous blood glucose monitoring using vascular catheter blood sampling is currently undergoing clinical trials and may become the standard of care and can allow tighter glycemic control in addition to preventing severe hypoglycemia or hyperglycemia[61]. Research has shown promise, as the technology is capable of detecting changes in glycemia that may otherwise be missed in our current practice, and has

Table 3 Strict glycemic control *vs* moderate glycemic control in critically ill patients with diabetes

Ref.	Study design/cohort	Sample size	Control group	Therapies employed	Conclusion	Favored therapy
Lecomte *et al*[57] (2011)	Diabetics undergoing off-pump cardiac bypass surgery	60	Matched 60 non-diabetics	Strict glycemic control (80-110 mg/dL)	Strict glycemic control was feasible and efficient Minimal risks for hypo- or hyperglycemia	Strict glycemic control
Yuan *et al*[58] (2015)	Diabetic patients receiving enteral nutrition after gastrectomy	212	None	Strict glycemic control (80-110 mg/dL) and moderate glycemic control (< 200 mg/dL)	Strict glycemic control lead to higher rates of severe hypoglycemia but lower rates of severe hyperglycemia Surgical site infection rate was higher with moderate glycemic control Rates of other complications were similar in the two groups	Strict glycemic control
Umpierrez *et al*[59] (2015)	Diabetic patients after coronary artery bypass surgery	152	150 non-diabetics	Strict glycemic control (100-140 mg/dL) and moderate glycemic control (141-180 mg/dL)	No significant differences between the two in the rate and severity of complications	Neither
Kar *et al*[9] (2016)	Diabetic ICU patients with HbA1c ⩾ 7.0% admission	83	None	Moderate glycemic control (< 180 mg/dL) and Loose glycemic control (< 250 mg/dL)	Loose glycemic control reduces glycemic variability and moderate to severe hypoglycemia	Loose glycemic control

ICU: Intensive care unit; HbA1c: Glycosylated hemoglobin.

shown that glucose levels correlate well with standard arterial glycemic measurement[62-64].

CONCLUSION

Glycemic control in the ICU continues to be challenging at best. Although the glycemic control strategy does not vary among diabetic individuals without persistent hyperglycemia from non-diabetic individuals (serum glucose goal of 140-180 mg/dL), it is important to note the cases where exceptions should be made. In neurological patients and surgical patients, a stricter glycemic strategy can be maintained (110-140 mg/dL and < 150 mg/dL, respectively) as long as adequate hypoglycemia can be avoided. In patients with a history of persistent hyperglycemia (HbA1c above 7%), liberal glycemic control may be beneficial in reducing the risk of hypoglycemia and glycemic variability, which is known to increase cardiovascular mortality, but further evidence and studies are necessary before a strong recommendation can be given. Further randomized control studies are suggested to further evaluate the variability in the target blood glucose level among different conditions.

REFERENCES

1 **Dungan KM**, Braithwaite SS, Preiser JC. Stress hyperglycaemia. *Lancet* 2009; **373**: 1798-1807 [PMID: 19465235 DOI: 10.1016/S0140-6736(09)60553-5]

2 **Bernard C**. Leèons sur le diabète et la glycogenèse animale. Paris: JB Bailliere et Fils, 1877

3 **Mesotten D**, Preiser JC, Kosiborod M. Glucose management in critically ill adults and children. *Lancet Diabetes Endocrinol* 2015; **3**: 723-733 [PMID: 26071884 DOI: 10.1016/S2213-8587(15)00223-5]

4 **Fahy BG**, Sheehy AM, Coursin DB. Glucose control in the intensive care unit. *Crit Care Med* 2009; **37**: 1769-1776 [PMID: 19325461 DOI: 10.1097/CCM.0b013e3181a19ceb]

5 **Monnier L**, Mas E, Ginet C, Michel F, Villon L, Cristol JP, Colette C. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA* 2006; **295**: 1681-1687 [PMID: 16609090 DOI: 10.1001/jama.295.14.1681]

6 **Falciglia M**, Freyberg RW, Almenoff PL, D'Alessio DA, Render ML. Hyperglycemia-related mortality in critically ill patients varies with admission diagnosis. *Crit Care Med* 2009; **37**: 3001-3009 [PMID: 19661802 DOI: 10.1097/CCM.0b013e3181b083f7]

7 **Centers for Disease Control and Prevention**. National Diabetes Statistics Report: Estimates of Diabetes and Its Burden in the United States, 2014. Atlanta, GA: US Department of Health and Human Services, 2014

8 **Fuchs L**, Chronaki CE, Park S, Novack V, Baumfeld Y, Scott D, McLennan S, Talmor D, Celi L. ICU admission characteristics and mortality rates among elderly and very elderly patients. *Intensive Care Med* 2012; **38**: 1654-1661 [PMID: 22797350 DOI: 10.1007/s00134-012-2629-6]

9 **Kar P**, Plummer MP, Bellomo R, Jenkins AJ, Januszewski AS, Chapman MJ, Jones KL, Horowitz M, Deane AM. Liberal Glycemic Control in Critically Ill Patients With Type 2 Diabetes: An Exploratory Study. *Crit Care Med* 2016; **44**: 1695-1703 [PMID: 27315191 DOI: 10.1097/CCM.0000000000001815]

10 **Goldstein DS**. Adrenal responses to stress. *Cell Mol Neurobiol* 2010; **30**: 1433-1440 [PMID: 21061156 DOI: 10.1007/s10571-010-9606-9]

11 **Herman JP**, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, Cullinan WE. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front Neuroendocrinol* 2003; **24**: 151-180 [PMID: 14596810]

12 **Chrousos GP**, Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA* 1992; **267**: 1244-1252 [PMID: 1538563]

13 **Marik PE**, Bellomo R. Stress hyperglycemia: an essential survival response! *Crit Care* 2013; **17**: 305 [PMID: 23470218 DOI: 10.1186/cc12514]

14 **Harp JB**, Yancopoulos GD, Gromada J. Glucagon orchestrates stress-induced hyperglycaemia. *Diabetes Obes Metab* 2016; **18**: 648-653 [PMID: 27027662 DOI: 10.1111/dom.12668]

15 **Viana MV**, Moraes RB, Fabbrin AR, Santos MF, Gerchman F. [Assessment and treatment of hyperglycemia in critically ill patients]. *Rev Bras Ter Intensiva* 2014; **26**: 71-76 [PMID: 24770692]

16 **van den Berghe G**, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R. Intensive insulin therapy in critically ill patients. *N Engl J Med* 2001; **345**: 1359-1367 [PMID: 11794168 DOI: 10.1056/NEJMoa011300]

17 **Van den Berghe G**, Wilmer A, Hermans G, Meersseman W, Wouters PJ, Milants I, Van Wijngaerden E, Bobbaers H, Bouillon R. Intensive insulin therapy in the medical ICU. *N Engl J Med* 2006; **354**: 449-461 [PMID: 16452557 DOI: 10.1056/NEJMoa052521]

18 **Finfer S**, Chittock DR, Su SY, Blair D, Foster D, Dhingra V, Bellomo R, Cook D, Dodek P, Henderson WR, Hébert PC, Heritier S, Heyland DK, McArthur C, McDonald E, Mitchell I, Myburgh JA, Norton R, Potter J, Robinson BG, Ronco JJ. Intensive versus conventional glucose control in critically ill patients. *N Engl J Med* 2009; **360**: 1283-1297 [PMID: 19318384 DOI: 10.1056/NEJMoa0810625]

19 **Marik PE**, Egi M. Treatment thresholds for hyperglycemia in critically ill patients with and without diabetes. *Intensive Care Med* 2014; **40**: 1049-1051 [PMID: 24859623 DOI: 10.1007/s00134-014-3344-2]

20 **Monnier L**, Colette C, Owens DR. Glycemic variability: the third component of the dysglycemia in diabetes. Is it important? How to measure it? *J Diabetes Sci Technol* 2008; **2**: 1094-1100 [PMID: 19885298]

21 **Krinsley JS**. Glycemic variability: a strong independent predictor of mortality in critically ill patients. *Crit Care Med* 2008; **36**: 3008-3013 [PMID: 18824908 DOI: 10.1097/CCM.0b013e31818b38d2]

22 **Krinsley JS**. Glycemic variability and mortality in critically ill patients: the impact of diabetes. *J Diabetes Sci Technol* 2009; **3**: 1292-1301 [PMID: 20144383]

23 **Landstedt-Hallin L**, Adamson U, Lins PE. Oral glibenclamide suppresses glucagon secretion during insulin-induced hypoglycemia in patients with type 2 diabetes. *J Clin Endocrinol Metab* 1999; **84**: 3140-3145 [PMID: 10487677 DOI: 10.1210/jcem.84.9.6002]

24 **Robinson RT**, Harris ND, Ireland RH, Macdonald IA, Heller SR. Changes in cardiac repolarization during clinical episodes of nocturnal hypoglycaemia in adults with Type 1 diabetes. *Diabetologia* 2004; **47**: 312-315 [PMID: 14712347 DOI: 10.1007/s00125-003-1292-4]

25 **Svensson AM**, McGuire DK, Abrahamsson P, Dellborg M. Association between hyper- and hypoglycaemia and 2 year all-cause mortality risk in diabetic patients with acute coronary events. *Eur Heart J* 2005; **26**: 1255-1261 [PMID: 15821004 DOI: 10.1093/eurheartj/ehi230]

26 **Kalfon P**, Le Manach Y, Ichai C, Bréchot N, Cinotti R, Dequin PF, Riu-Poulenc B, Montravers P, Annane D, Dupont H, Sorine M, Riou B. Severe and multiple hypoglycemic episodes are associated with increased risk of death in ICU patients. *Crit Care* 2015; **19**: 153 [PMID: 25888011 DOI: 10.1186/s13054-015-0851-7]

27 **Saisho Y**. Glycemic variability and oxidative stress: a link between diabetes and cardiovascular disease? *Int J Mol Sci* 2014; **15**: 18381-18406 [PMID: 25314300 DOI: 10.3390/ijms151018381]

28 Professional Practice Committee for the Standards of Medical Care in Diabetes-2016. *Diabetes Care* 2016; **39** Suppl 1: S107-S108 [PMID: 26696673 DOI: 10.2337/dc16-S018]

29 **Moghissi ES**, Korytkowski MT, DiNardo M, Einhorn D, Hellman R, Hirsch IB, Inzucchi SE, Ismail-Beigi F, Kirkman MS, Umpierrez GE. American Association of Clinical Endocrinologists and American Diabetes Association consensus statement on inpatient glycemic control. *Diabetes Care* 2009; **32**: 1119-1131 [PMID: 19429873 DOI: 10.2337/dc09-9029]

30 **Wiener RS**, Wiener DC, Larson RJ. Benefits and risks of tight glucose control in critically ill adults: a meta-analysis. *JAMA* 2008; **300**: 933-944 [PMID: 18728267 DOI: 10.1001/jama.300.8.933]

31 **Griesdale DE**, de Souza RJ, van Dam RM, Heyland DK, Cook DJ, Malhotra A, Dhaliwal R, Henderson WR, Chittock DR, Finfer S, Talmor D. Intensive insulin therapy and mortality among critically ill patients: a meta-analysis including NICE-SUGAR study data. *CMAJ* 2009; **180**: 821-827 [PMID: 19318387 DOI: 10.1503/cmaj.090206]

32 **Qaseem A**, Chou R, Humphrey LL, Shekelle P. Inpatient glycemic control: best practice advice from the Clinical Guidelines Committee of the American College of Physicians. *Am J Med Qual* 2014; **29**: 95-98 [PMID: 23709472 DOI: 10.1177/1062860613489339]

33 **Jacobi J**, Bircher N, Krinsley J, Agus M, Braithwaite SS, Deutschman C, Freire AX, Geehan D, Kohl B, Nasraway SA, Rigby M, Sands K, Schallom L, Taylor B, Umpierrez G, Mazuski J, Schunemann H. Guidelines for the use of an insulin infusion for the management of hyperglycemia in critically ill patients. *Crit Care Med* 2012; **40**: 3251-3276 [PMID: 23164767 DOI: 10.1097/CCM.0b013e3182653269]

34 **Egi M**, Bellomo R, Stachowski E, French CJ, Hart GK, Taori G, Hegarty C, Bailey M. The interaction of chronic and acute glycemia with mortality in critically ill patients with diabetes. *Crit Care Med* 2011; **39**: 105-111 [PMID: 20975552 DOI: 10.1097/CCM.0b013e3181feb5ea]

35 **Plummer MP**, Bellomo R, Cousins CE, Annink CE, Sundararajan K, Reddi BA, Raj JP, Chapman MJ, Horowitz M, Deane AM. Dysglycaemia in the critically ill and the interaction of chronic and acute glycaemia with mortality. *Intensive Care Med* 2014; **40**: 973-980 [PMID: 24760120 DOI: 10.1007/s00134-014-3287-7]

36 **Egi M**, Bellomo R, Stachowski E, French CJ, Hart GK, Hegarty C, Bailey M. Blood glucose concentration and outcome of critical illness: the impact of diabetes. *Crit Care Med* 2008; **36**: 2249-2255 [PMID: 18664780 DOI: 10.1097/CCM.0b013e318181039a]

37 **Krinsley JS**, Egi M, Kiss A, Devendra AN, Schuetz P, Maurer PM, Schultz MJ, van Hooijdonk RT, Kiyoshi M, Mackenzie IM, Annane D, Stow P, Nasraway SA, Holewinski S, Holzinger U, Preiser JC, Vincent JL, Bellomo R. Diabetic status and the relation of the three domains of glycemic control to mortality in critically ill patients: an international multicenter cohort study. *Crit Care* 2013; **17**: R37 [PMID: 23452622 DOI: 10.1186/cc12547]

38 **Dungan KM**. Hyperglycemia in the intensive care unit: is insulin the only option? *Crit Care* 2013; **17**: 1012 [PMID: 25169675 DOI: 10.1186/cc13107]

39 **Lazar HL**, McDonnell M, Chipkin SR, Furnary AP, Engelman RM, Sadhu AR, Bridges CR, Haan CK, Svedjeholm R, Taegtmeyer H, Shemin RJ. The Society of Thoracic Surgeons practice guideline series: Blood glucose management during adult cardiac surgery. *Ann Thorac Surg* 2009; **87**: 663-669 [PMID: 19161815 DOI: 10.1016/j.athoracsur.2008.11.011]

40 **Furnary AP**, Wu Y, Bookin SO. Effect of hyperglycemia and continuous intravenous insulin infusions on outcomes of cardiac surgical procedures: the Portland Diabetic Project. *Endocr Pract* 2004; **10** Suppl 2: 21-33 [PMID: 15251637 DOI: 10.4158/EP.10.S2.21]

41 **Okabayashi T**, Shima Y, Sumiyoshi T, Kozuki A, Tokumaru T, Iiyama T, Sugimoto T, Kobayashi M, Yokoyama M, Hanazaki K. Intensive versus intermediate glucose control in surgical intensive care unit patients. *Diabetes Care* 2014; **37**: 1516-1524 [PMID: 24623024 DOI: 10.2337/dc13-1771]

42 **Bilotta F**, Caramia R, Paoloni FP, Delfini R, Rosa G. Safety and efficacy of intensive insulin therapy in critical neurosurgical patients. *Anesthesiology* 2009; **110**: 611-619 [PMID: 19237874 DOI: 10.1097/ALN.0b013e318198004b]

43 **Kramer AH**, Roberts DJ, Zygun DA. Optimal glycemic control in neurocritical care patients: a systematic review and meta-analysis. *Crit Care* 2012; **16**: R203 [PMID: 23082798 DOI: 10.1186/cc11812]

44 **Deedwania P**, Kosiborod M, Barrett E, Ceriello A, Isley W, Mazzone T, Raskin P. Hyperglycemia and acute coronary syndrome: a scientific statement from the American Heart Association Diabetes Committee of the Council on Nutrition, Physical Activity, and Metabolism. *Circulation* 2008; **117**: 1610-1619 [PMID: 18299505 DOI: 10.1161/CIRCULATIONAHA.107.188629]

45 **Kushner FG**, Hand M, Smith SC, King SB, Anderson JL, Antman EM, Bailey SR, Bates ER, Blankenship JC, Casey DE, Green LA, Hochman JS, Jacobs AK, Krumholz HM, Morrison DA, Ornato JP, Pearle DL, Peterson ED, Sloan MA, Whitlow PL, Williams DO. 2009 Focused Updates: ACC/AHA Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction (updating the

2004 Guideline and 2007 Focused Update) and ACC/AHA/SCAI Guidelines on Percutaneous Coronary Intervention (updating the 2005 Guideline and 2007 Focused Update): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation* 2009; **120**: 2271-2306 [PMID: 19923169 DOI: 10.1161/CIRCULATIONAHA.109.192663]

46 **Steg PG**, James SK, Atar D, Badano LP, Blömstrom-Lundqvist C, Borger MA, Di Mario C, Dickstein K, Ducrocq G, Fernandez-Aviles F, Gershlick AH, Giannuzzi P, Halvorsen S, Huber K, Juni P, Kastrati A, Knuuti J, Lenzen MJ, Mahaffey KW, Valgimigli M, van 't Hof A, Widimsky P, Zahger D. ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation. *Eur Heart J* 2012; **33**: 2569-2619 [PMID: 22922416 DOI: 10.1093/eurheartj/ehs215]

47 **Centre for Clinical Practice at NICE (UK)**. Hyperglycaemia in Acute Coronary Syndromes: Management of Hyperglycaemia in People with Acute Coronary Syndromes. London: National Institute for Health and Clinical Excellence (UK), 2011 [PMID: 23346606]

48 **Chatterjee S**, Sharma A, Lichstein E, Mukherjee D. Intensive glucose control in diabetics with an acute myocardial infarction does not improve mortality and increases risk of hypoglycemia-a meta-regression analysis. *Curr Vasc Pharmacol* 2013; **11**: 100-104 [PMID: 22724474]

49 **Ellger B**, Westphal M, Stubbe HD, Van den Heuvel I, Van Aken H, Van den Berghe G. [Glycemic control in sepsis and septic shock: friend or foe?]. *Anaesthesist* 2008; **57**: 43-48 [PMID: 18034219 DOI: 10.1007/s00101-007-1285-7]

50 **Martin-Loeches I**, Levy MM, Artigas A. Management of severe sepsis: advances, challenges, and current status. *Drug Des Devel Ther* 2015; **9**: 2079-2088 [PMID: 25926718 DOI: 10.2147/DDDT.S78757]

51 **Dellinger RP**, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb S, Beale RJ, Vincent JL, Moreno R. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* 2013; **39**: 165-228 [PMID: 23361625 DOI: 10.1007/s00134-012-2769-8]

52 **Crowther CA**, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N Engl J Med* 2005; **352**: 2477-2486 [PMID: 15951574 DOI: 10.1056/NEJMoa042973]

53 **Baz B**, Riveline JP, Gautier JF. Endocrinology of pregnancy: Gestational diabetes mellitus: definition, aetiological and clinical aspects. *Eur J Endocrinol* 2016; **174**: R43-R51 [PMID: 26431552 DOI: 10.1530/EJE-15-0378]

54 **Catalano PM**. Trying to understand gestational diabetes. *Diabet Med* 2014; **31**: 273-281 [PMID: 24341419 DOI: 10.1111/dme.12381]

55 **Van de Velde MSH**, Plante, L. Maternal Critical Care. Cambridge University Press, The Edinburgh Building, Cambridge CB2 8RU, United Kingdom: Cambridge University Press, 2013

56 **Metzger BE**, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, Hadden DR, McCance DR, Hod M, McIntyre HD, Oats JJ, Persson B, Rogers MS, Sacks DA. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008; **358**: 1991-2002 [PMID: 18463375 DOI: 10.1056/NEJMoa0707943]

57 **Lecomte P**, Foubert L, Coddens J, Dewulf B, Nobels F, Casselman F, Cammu G. Management of tight intraoperative glycemic control during off-pump coronary artery bypass surgery in diabetic and nondiabetic patients. *J Cardiothorac Vasc Anesth* 2011; **25**: 937-942 [PMID: 21640613 DOI: 10.1053/j.jvca.2011.03.173]

58 **Yuan J**, Liu T, Zhang X, Si Y, Ye Y, Zhao C, Wang Q, Shen X. Intensive Versus Conventional Glycemic Control in Patients with Diabetes During Enteral Nutrition After Gastrectomy. *J Gastrointest Surg* 2015; **19**: 1553-1558 [PMID: 26084869 DOI: 10.1007/s11605-015-2871-7]

59 **Umpierrez G**, Cardona S, Pasquel F, Jacobs S, Peng L, Unigwe M, Newton CA, Smiley-Byrd D, Vellanki P, Halkos M, Puskas JD, Guyton RA, Thourani VH. Randomized Controlled Trial of Intensive Versus Conservative Glucose Control in Patients Undergoing Coronary Artery Bypass Graft Surgery: GLUCO-CABG Trial. *Diabetes Care* 2015; **38**: 1665-1672 [PMID: 26180108 DOI: 10.2337/dc15-0303]

60 **Clement S**, Braithwaite SS, Magee MF, Ahmann A, Smith EP, Schafer RG, Hirsch IB. Management of diabetes and hyperglycemia in hospitals. *Diabetes Care* 2004; **27**: 553-591 [PMID: 14747243]

61 **Joseph JI**, Torjman MC, Strasma PJ. Vascular Glucose Sensor Symposium: Continuous Glucose Monitoring Systems (CGMS) for Hospitalized and Ambulatory Patients at Risk for Hyperglycemia, Hypoglycemia, and Glycemic Variability. *J Diabetes Sci Technol* 2015; **9**: 725-738 [PMID: 26078254 DOI: 10.1177/19322968155 87938]

62 **De Block C**, Manuel-Y-Keenoy B, Van Gaal L, Rogiers P. Intensive insulin therapy in the intensive care unit: assessment by continuous glucose monitoring. *Diabetes Care* 2006; **29**: 1750-1756 [PMID: 16873775 DOI: 10.2337/dc05-2353]

63 **De Block C**, Manuel-y-Keenoy B, Rogiers P, Jorens P, Van Gaal L. Glucose control and use of continuous glucose monitoring in the intensive care unit: a critical review. *Curr Diabetes Rev* 2008; **4**: 234-244 [PMID: 18690906]

64 **De Block CE**, Gios J, Verheyen N, Manuel-y-Keenoy B, Rogiers P, Jorens PG, Scuffi C, Van Gaal LF. Randomized Evaluation of Glycemic Control in the Medical Intensive Care Unit Using Real-Time Continuous Glucose Monitoring (REGIMEN Trial). *Diabetes Technol Ther* 2015; **17**: 889-898 [PMID: 26305390 DOI: 10.1089/dia.2015.0151]

65 **Hsu CW**. Glycemic control in critically ill patients. *World J Crit Care Med* 2012; **1**: 31-39 [PMID: 24701399 DOI: 10.5492/wjccm.v1.i1.31]

66 **Finfer S**, Liu B, Chittock DR, Norton R, Myburgh JA, McArthur C, Mitchell I, Foster D, Dhingra V, Henderson WR, Ronco JJ, Bellomo R, Cook D, McDonald E, Dodek P, Hébert PC, Heyland DK, Robinson BG. Hypoglycemia and risk of death in critically ill patients. *N Engl J Med* 2012; **367**: 1108-1118 [PMID: 22992074 DOI: 10.1056/NEJMoa1204942]

P- Reviewer: Malfitano C, Kumar KVSH, Pastromas S
S- Editor: Kong JX **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/ *World J Diabetes* 2017 March 15; 8(3): 97-103

DOI: 10.4239/wjd.v8.i3.97 ISSN 1948-9358 (online)

MINIREVIEWS

Involvement of Cbl-b-mediated macrophage inactivation in insulin resistance

Tomoki Abe, Katsuya Hirasaka, Takeshi Nikawa

Tomoki Abe, Takeshi Nikawa, Department of Nutritional Physiology, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima 770-8503, Japan

Katsuya Hirasaka, Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, Nagasaki 852-8521, Japan

Author contributions: Abe T generated the figures and wrote the manuscript; Hirasaka K and Nikawa T specified the aims of the editorial and assisted in writing the manuscript.

Supported by The Japan Society for the Promotion of Science KAKENHI (to Tomoki Abe), No. JP15K16208.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Manuscript source: Invited manuscript

Correspondence to: Takeshi Nikawa, MD, PhD, Professor, Department of Nutritional Physiology, Institute of Biomedical Sciences, Tokushima University Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan. nikawa@tokushima-u.ac.jp
Telephone: +81-88-6339248
Fax: +81-88-6337086

Received: March 25, 2016
Peer-review started: March 26, 2016
First decision: May 13, 2016
Revised: December 23, 2016
Accepted: January 11, 2017
Article in press: January 13, 2017
Published online: March 15, 2017

Abstract

Aging and overnutrition cause obesity in rodents and humans. It is well-known that obesity causes various diseases by producing insulin resistance (IR). Macrophages infiltrate the adipose tissue (AT) of obese individuals and cause chronic low-level inflammation associated with IR. Macrophage infiltration is regulated by the chemokines that are released from hypertrophied adipocytes and the immune cells in AT. Saturated fatty acids are recognized by toll-like receptor 4 (TLR4) and induce inflammatory responses in AT macrophages (ATMs). The inflammatory cytokines that are released from activated ATMs promote IR in peripheral organs, such as the liver, skeletal muscle and AT. Therefore, ATM activation is a therapeutic target for IR in obesity. The ubiquitin ligase Casitas b-lineage lymphoma-b (Cbl-b) appears to potently suppress macrophage migration and activation. Cbl-b is highly expressed in leukocytes and negatively regulates signals associated with migration and activation. Cbl-b deficiency enhances ATM accumulation and IR in aging- and diet-induced obese mice. Cbl-b inhibits migration-related signals and SFA-induced TLR4 signaling in ATMs. Thus, targeting Cbl-b may be a potential therapeutic strategy to reduce the IR induced by ATM activation. In this review, we summarize the regulatory functions of Cbl-b in ATMs.

Key words: Casitas b-lineage lymphoma-b; Insulin resistance; Macrophage; Obesity; Toll-like receptor 4

Core tip: Obesity leads to the development of chronic inflammation and insulin resistance (IR). Adipose tissue macrophages (ATMs) play a crucial role in the development of obesity-induced IR. Therefore, ATMs are attractive therapeutic targets for IR. Recently, we demonstrated that the ubiquitin ligase Casitas b-lineage lymphoma-b (Cbl-b) negatively regulates the migration and activation of ATMs.

Here, we review key aspects of Cbl-b function in the regulation of ATMs.

Abe T, Hirasaka K, Nikawa T. Involvement of Cbl-b-mediated macrophage inactivation in insulin resistance. *World J Diabetes* 2017; 8(3): 97-103 Available from: URL: http://www.wjgnet.com/1948-9358/full/v8/i3/97.htm DOI: http://dx.doi.org/10.4239/wjd.v8.i3.97

INTRODUCTION

In 2014, more than 1.9 billion adults were overweight, and of these, over 600 million were obese[1]. Obesity is a risk factor for the development of insulin resistance (IR), diabetes mellitus, hepatic steatosis and hypertension[2], resulting in escalating healthcare costs in several developed countries. Thus, it is important to elucidate the mechanism for obesity-associated IR and develop attractive therapeutic strategies for treating IR. A combination of various factors, such as diet, lifestyle, genetic background, psychological stress and aging, leads to obesity. In particular, aging and nutritional excess play critical roles in the development of obesity.

Aging causes decreases in physical activity, lean body mass and anti-oxidant defenses, thus increasing oxidative stress and the number of damaged cells[3]. These changes are associated with lipid accumulation in white adipose tissue (WAT) due to decreased energy expenditure. The oxidative stress induced by aging causes mitochondrial dysfunction and muscle atrophy. Sarcopenia, aging-induced skeletal muscle loss, decreases energy expenditures and causes obesity[4]. An excessive intake of carbohydrates and lipids causes the accumulation of triacylglycerols in adipocytes, which produces expansion of the adipocyte. Obesity causes inflammatory responses in WAT. It is well-known that in addition to its roles in fat storage, AT also plays key roles in endocrine system. AT secretes lipids, adipokines and chemokines to maintain homeostasis. The hypertrophy of the AT alters adipokine and chemokine secretion[2,5]. It is well-known that diverse immune cells reside in WAT of both lean and obese individuals, and these cells release inflammatory cytokines during obesity. Resident eosinophils and regulatory $CD4^+$ helper T cells maintain homeostasis in the AT of lean subjects[6]. In contrast to $CD4^+$ T cells, $CD8^+$ T cells increase in number in the AT of obese subjects and promote the inflammatory responses mediated by macrophages[7]. AT macrophages (ATMs) also release various inflammatory mediators. Because ATMs play a key role in obesity-associated inflammatory action, the suppression of ATM activation is a potent therapeutic strategy for treating IR induced by obesity. Recently, several studies demonstrated that the ubiquitin ligase Casitas b-lineage lymphoma-b (Cbl-b) is a key regulator of macrophage activation[8-10]. Here, we review the key roles of Cbl-b in ATM activation and the pathogenesis of IR in obesity.

THE UBIQUITIN LIGASE CBL-B

In mammalian cells, there are three major intracellular protein degradation pathways. The calpain pathway, the autophagy-lysosome pathway, and the ubiquitin (Ub)-proteasome system play important roles in maintaining cellular homeostasis. In particular, the Ub-proteasome system is regulated by three types of enzymes: A Ub-activating enzyme (E1), a Ub-conjugating enzyme (E2) and a Ub ligase (E3). In the initial step, the activation of Ub proteins by E1 enzymes is critically dependent on the presence of ATP. An E1 enzyme transfers a Ub protein to E2 enzyme. And then, the E2 enzymes shuttle a Ub protein to an E3 enzyme, which ubiquitinates the specific target protein. The proteins tagged with Ub are specifically degraded by the proteasome. Therefore, E3 enzymes are important for determining the specific target proteins that will be degraded by proteasome[11].

The Cbl proteins in mammalian (c-Cbl, Cbl-b and Cbl-c), which were originally identified as adaptor molecules, function as ubiquitin ligases (Figure 1). A number of studies show that Cbl proteins inhibit the signal transduction by receptor and non-receptor tyrosine kinases[12-14]. The protein tyrosine kinase-binding (TKB) and really interesting new gene (RING) finger (RF) domains are highly conserved in the N-terminal domains of all Cbl homologues. The TKB domain, which is a specific domain in Cbl proteins, binds to the phosphorylated tyrosines of the substrates through Src-homology (SH) 2 domains[15]. The RF catalytic domain has the E3 ubiquitin ligase activity because it binds to E2 enzymes[16]. Cbl-b is a substrate of tyrosine kinases, and the ubiquitin ligase activity is regulated by the phosphorylation of some tyrosine residues[14,17,18]. Increasing evidence indicates that Cbl-b is abundantly expressed in leukocytes and decreases the activation of various immune cells. Therefore, loss-of-function mutations of *Cblb* cause various autoimmune diseases[19-21]. Interestingly, a *Cblb* mutation was identified as factor associated with diabetes in a rat model of human type Ⅰ diabetes[20,22]. Yokoi *et al*[22] reported that F328L is a loss-of-function mutation in T cells that was identified in Japanese subjects. These studies reveal that the function of Cbl-b is connected to diabetes.

INFLAMMATORY ACTIONS OF MACROPHAGES IN ADIPOSE TISSUE

Various immune cells, such as macrophages, T cells, mast cells, natural killer cells and eosinophils, reside in WAT along with adipocytes. The expansion of adipocytes alters these populations in WAT. ATMs increase the number of cells in the AT of obese mice[23]. ATMs play important roles in the AT of lean and obese humans and rodents. In the AT of lean subjects, resident M2-like or alternatively activated ATMs preferentially maintain homeostasis by secreting anti-inflammatory cytokines. In contrast, in obesity, the M1-like or classically activated ATMs in WAT induce inflammation mediated by the release of inflammatory cytokines and

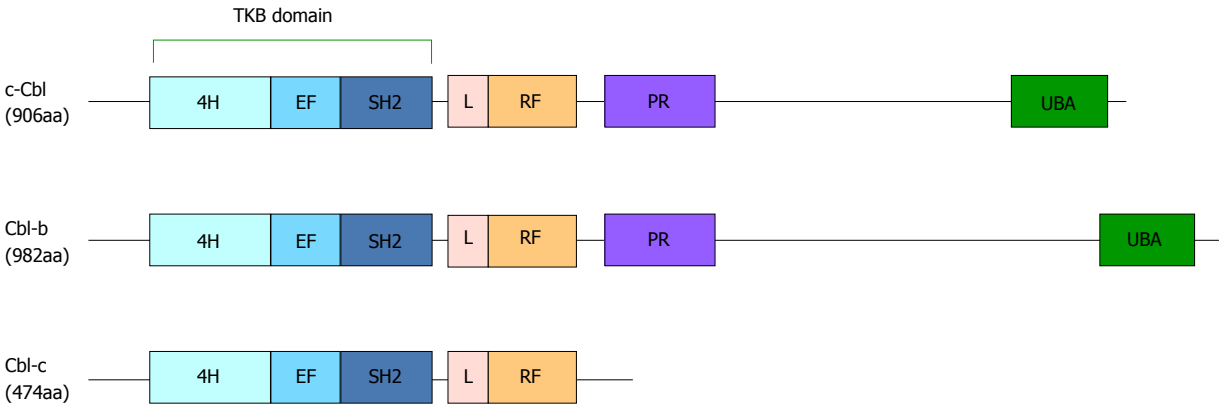


Figure 1 The primary structure and domain organization of human Casitas b-lineage lymphoma family proteins. Cbl-b proteins contain highly conserved tyrosine kinase-binding (TKB), linker (L), RING finger (RF) and proline-rich (PR) domains. 4H: Four-helix bundle; SH2: Src-homology 2; UBA: Ubiquitin-associated domain.

chemokines. ATMs are activated by saturated fatty acids (SFAs) through toll-like receptor 4 (TLR4). Although TLR4 was identified as the receptor for lipopolysaccharide (LPS), which is a component of the outer membrane of gram-negative bacteria[24], SFAs also activate TLR4 signaling in macrophages. The global mutation or the bone marrow-specific deficiency of TLR4 abrogated the systemic IR induced by the consumption of a high-fat diet (HFD)[25-27]. However, the molecular mechanism of TLR4 activation by SFAs is poorly understood. It is thought that SFAs fail to directly bind to TLR4[28]. A recent study[29] showed that SFAs activate the TLR4 signaling mediated by fetuin-A, a 64 kDa glycoprotein released from the liver in response to HFD consumption. Fetuin-A mediates SFA-induced activation of TLR4 by directly interacting with TLR4 in macrophages and adipocytes[29]. Interestingly, treatment with the insulin sensitizer pioglitazone suppresses fetuin-A expression through peroxisome proliferator-activated receptor-γ activation in hepatoma cells[30]. SFA treatments induce the activation of nuclear factor κB (NF-κB) and Jun N-terminal kinase (JNK), which are TLR4 signaling molecules in macrophages[26,31]. In fact, the inhibition of NF-κB or JNK ameliorates IR by activating ATMs in obese rodents[32,33]. Therefore, the regulation of ATM activation is a potent therapeutic target for obesity-associated IR.

CBL-B IN ATM RECRUITMENT

Aging and overnutrition cause the hypertrophy of AT, resulting in the accumulation of ATMs[5]. The activated ATMs induce peripheral and systemic IR through the release of inflammatory cytokines. JNK is a TLR4 signaling molecule and mediates the expression of inflammatory cytokines in macrophages. Bone marrow-specific deficiency of JNK1 ameliorated diet-induced IR by suppressing AT inflammation in mice[34]. We demonstrated that depletion of Cbl-b exacerbated obesity and IR induced by aging and HFD in mice[35,36]. We also found that ATM activation was enhanced in Cbl-b knockout (Cbl-b$^{-/-}$) mice. In 30-wk old Cbl-b$^{-/-}$ mice, we observed hypertrophy of AT, IR, hepatic steatosis and β cell dysfunction (Table 1). Interestingly, the ATM accumulation was dramatically increased in WAT. This event was caused by two factors in Cbl-b$^{-/-}$ mice. One factor was the high levels of monocyte chemotactic protein (MCP)-1/CC chemokine ligand 2 protein in circulation and WAT. MCP-1 is a member of CC chemokines, and causes the chemotaxis of leukocytes[37]. Previous reports demonstrated that MCP-1 and CC chemokine receptor type 2 (CCR2), the receptor for MCP-1, are associated with obesity-induced IR, inflammation and ATM accumulation[38-41]. In addition, CCR2 causes hepatic infiltration of macrophages and steatosis in mice[42,43]. Taken together, the data indicate that the inhibition of CCR2 is a potent therapeutic strategy for treating obesity-induced inflammation and IR.

Furthermore, it is known that Cbl-b decreases the migration-related signaling in macrophages. Macrophage migration is regulated by activation of the guanine nucleotide exchange factor Vav1[44]. Previous studies demonstrated that phosphorylation of Vav1 at Tyr267 mediated by spleen tyrosine kinase (Syk) is critical for Vav1 activation in leukocytes[45,46]. Cbl-b directly binds to Vav1 in T cells[47,48]. Although Vav1 phosphorylation is inhibited by Cbl-b, Cbl-b does not induce the degradation of Vav1. We also demonstrated that the depletion of Cbl-b promoted tyrosine phosphorylation in Vav1 in peritoneal macrophages from mice. These results indicated that the increased MCP-1 released from WAT and Vav1 phosphorylation cause ATM accumulation in Cbl-b$^{-/-}$ mice (Figure 2). In fact, treatment with an anti-MCP-1 antibody reduced the IR and ATM accumulation in Cbl-b$^{-/-}$ mice. Thus, Cbl-b may serve as a therapeutic target to reduce the IR mediated by the accumulation of ATMs.

CBL-B IN TLR4 SIGNALING

Several ubiquitin ligases have been identified as negative regulators of TLR4 signaling[49-52]. Triad3A is a RF ubiquitin ligase and directly binds to TLR4, resulting in ubiquitination and proteolytic degradation. Recent reports indicate that TLR4 signaling is inhibited by Cbl-b in macrophages and neutrophils[8,53]. Han *et al*[8] demonstrated that TLR4 signaling induced by LPS was suppressed in macrophages

Table 1 Phenotypes of Cbl-b$^{-/-}$ mice

Age and diet	Phenotypes	Ref.
30-wk old, normal diet	Adipose tissue inflammation Adiposity Fasting hyperinsulinemia Hepatic steatosis Impaired glucose tolerance Insulin resistance	[35]
13-wk old, high-fat diet	Adipose tissue inflammation Adiposity Fasting hyperleptinemia Fasting hyperlipidemia Fasting hypoadiponectinemia Insulin resistance	[36]

by Cbl-b-mediated ubiquitination and breakdown of toll/IL-1 receptor domain-containing adapter inducing interferon-β (TRIF) and MyD88, which are adaptor molecules for TLR4 signal transduction. This suppression by Cbl-b was dependent on the presence of integrin α_M (CD11b). In neutrophils, Cbl-b also suppresses LPS signaling by preventing the formation of the TLR4-MyD88 complex[53]. These reports suggest that Cbl-b is a critical regulator of the macrophage activation mediated by LPS-induced TLR4 signaling.

TLR4 activation by SFAs thought to play a pivotal role in ATM activation-induced IR. Diet-induced obesity increases the circulating levels of free FAs. SFAs directly induce IR in the liver, skeletal muscle and AT[54]. Furthermore, SFAs cause chronic inflammation through ATM activation, which is mediated by TLR4 signal transduction[25,26]. Recently, we demonstrated that the knockout of Cbl-b promoted and IR through ATM accumulation in HFD-fed mice[36]. In addition to increased ATM accumulation, inflammatory cytokine secretion was increased in the AT of obese Cbl-b$^{-/-}$ mice. In addition to aging, the consumption of a HFD increases MCP-1 expression in WAT. We found that depletion of Cbl-b in murine peritoneal macrophages promotes SFA-induced TLR4 signal transduction (Figure 3). Palmitate-induced JNK phosphorylation and IL-6 expression were enhanced in Cbl-b-deficient peritoneal macrophages. We also showed that TLR4 is a substrate for Cbl-b in the presence of SFAs. Overexpression of Cbl-b increased the ubiquitination and breakdown of TLR4 after palmitate treatment. Consistent with this finding, the TLR4 protein expression levels on the surface of Cbl-b-deficient peritoneal macrophages were increased. It is well known that LPS treatment induces the phosphorylation of 2 tyrosine residues of human TLR4[55]. The phosphorylation of TLR4 is required to activate signaling by promoting an interaction with Syk in macrophages[56]. It remains unknown whether SFAs also induce the TLR4 tyrosine phosphorylation in macrophages. Although LPS induces the ubiquitination and degradation of MyD88 and TRIF[8], SFAs do not induce these pathways in macrophages[36]. These differences between LPS and SFAs are not fully understood. Further investigations are needed to elucidate the mechanism of SFA-induced phosphorylation of TLR4.

Recently, Lu *et al*[57] reported that treatment with a TLR4 antagonist improves insulin sensitivity and macrophage accumulation in the atherosclerotic lesions of low-density lipoprotein receptor-deficient mice. We demonstrated the TLR4 signaling was strongly associated with the development of IR in obese Cbl-b$^{-/-}$ mice using eritoran, a TLR4 antagonist[58]. The eritoran treatment reduced the insulin sensitivity and glucose tolerance in obese Cbl-b$^{-/-}$ mice. This phenomenon may be caused by a decrease in ATM accumulation. In fact, we found that an anti-TLR4 antibody inhibited SFA-induced TLR4 signal transduction in murine peritoneal macrophages. Our data suggest that TLR4 antagonists are potent therapeutic drugs that can be used to treat the IR mediated by ATM activation.

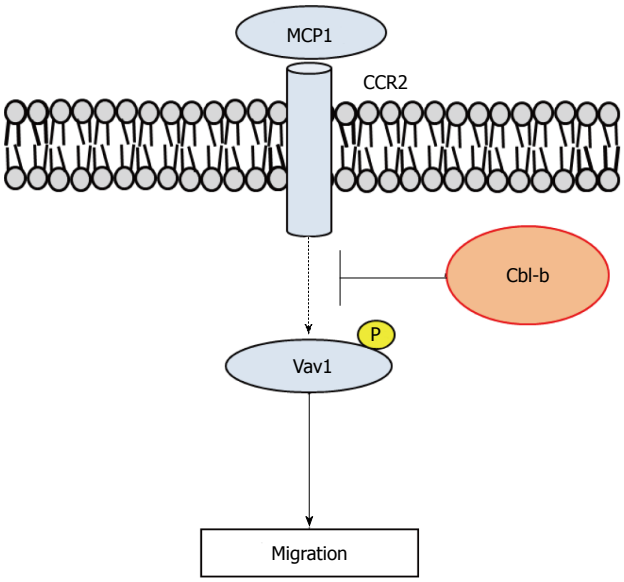


Figure 2 Casitas b-lineage lymphoma-b suppresses macrophage migration. Monocyte chemoattractant protein (MCP)-1 causes macrophages to infiltrate adipose tissue *via* C-C chemokine receptor 2 (CCR2). Phosphorylation (P) of Vav1 mediates macrophage migration, and Cbl-b negatively regulates macrophage migration by suppressing Vav1 phosphorylation.

CONCLUSION

Obesity causes various diseases through the development of IR, which is a clinical feature of patients with type 2 diabetes. Prediabetes is defined as impaired fasting glucose, impaired glucose tolerance and/or high levels of plasma glycated hemoglobin and is a critical risk factor for cardiovascular diseases[59]. AT inflammation is thought to be associated with the onset of prediabetes[60]. Therefore, to prevent type 2 diabetes, the development of ab effective therapeutic strategy for obesity-induced IR is urgently needed.

Aging- and diet-induced obesity causes the IR mediated by ATM activation. However, the mechanisms underlying ATM activation are poorly understood. We showed that Cbl-b reduces IR by suppressing macrophage migration and activation in mice. However, several questions remain about the biological implication of Cbl-b in human cells. The molecular mechanism underlying the effects of Cbl-b

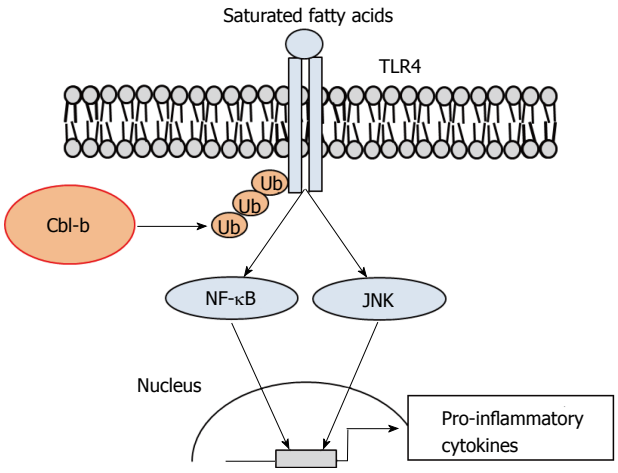


Figure 3 Casitas b-lineage lymphoma-b suppresses toll-like receptor 4 signaling in macrophages. Cbl-b negatively regulates saturated fatty acid (SFA)-induced TLR4 signal transduction. SFA-triggered TLR4 signaling induces the expression of inflammatory cytokines *via* JNK and NF-κB. The released inflammatory cytokines cause insulin resistance in the liver, skeletal muscle and adipose tissue. In the presence of SFAs, Cbl-b induces the ubiquitination and degradation of TLR4 in macrophages. Ub: Ubiquitin; TLR4: Toll-like receptor 4; Cbl-b: Casitas b-lineage lymphoma-b; JNK: Jun N-terminal kinase.

in macrophages is unknown. Further investigations are essential to identify new tyrosine kinases for Cbl-b. Recently, it was shown that macrophages infiltrate the fatty liver and AT in obesity. Cbl-b may suppress the macrophage activation in fatty liver. The side effects of Cbl-b activation remain unclear. We also showed that Cbl-b disturbed insulin-like growth factor signaling through ubiquitination and degradation of insulin receptor substrate-1 in skeletal muscle under unloading conditions[61]. Although we did not observe an enhancement of insulin signal transduction in lean Cbl-b$^{-/-}$ mice, tissue-specific Cbl-b activation may be important when using a drug delivery system, such as liposomes. A better understanding of Cbl-b-mediated ATM activation may provide the basis for developing novel therapeutic strategies that can be used to treat IR.

REFERENCES

1 **WHO**. World Health Organization. Obesity and Overweight, 2015 [Online] 2015. Available from: URL: http://www.who.int/mediacentre/factsheets/fs311/en/

2 **Wellen KE**, Thompson CB. Cellular metabolic stress: considering how cells respond to nutrient excess. *Mol Cell* 2010; **40**: 323-332 [PMID: 20965425 DOI: 10.1016/j.molcel.2010.10.004]

3 **Reinisalo M**, Kårlund A, Koskela A, Kaarniranta K, Karjalainen RO. Polyphenol Stilbenes: Molecular Mechanisms of Defence against Oxidative Stress and Aging-Related Diseases. *Oxid Med Cell Longev* 2015; **2015**: 340520 [PMID: 26180583 DOI: 10.1155/2015/340520]

4 **Karakelides H**, Nair KS. Sarcopenia of aging and its metabolic impact. *Curr Top Dev Biol* 2005; **68**: 123-148 [PMID: 16124998 DOI: 10.1016/S0070-2153(05)68005-2]

5 **Barzilai N**, Huffman DM, Muzumdar RH, Bartke A. The critical role of metabolic pathways in aging. *Diabetes* 2012; **61**: 1315-1322 [PMID: 22618766 DOI: 10.2337/db11-1300]

6 **Lee BC**, Lee J. Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance. *Biochim Biophys Acta* 2014; **1842**: 446-462 [PMID: 23707515 DOI: 10.1016/j.bbadis.2013.05.017]

7 **Nishimura S**, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, Otsu M, Hara K, Ueki K, Sugiura S, Yoshimura K, Kadowaki T, Nagai R. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat Med* 2009; **15**: 914-920 [PMID: 19633658 DOI: 10.1038/nm.1964]

8 **Han C**, Jin J, Xu S, Liu H, Li N, Cao X. Integrin CD11b negatively regulates TLR-triggered inflammatory responses by activating Syk and promoting degradation of MyD88 and TRIF via Cbl-b. *Nat Immunol* 2010; **11**: 734-742 [PMID: 20639876 DOI: 10.1038/ni.1908]

9 **Zhu LL**, Luo TM, Xu X, Guo YH, Zhao XQ, Wang TT, Tang B, Jiang YY, Xu JF, Lin X, Jia XM. E3 ubiquitin ligase Cbl-b negatively regulates C-type lectin receptor-mediated antifungal innate immunity. *J Exp Med* 2016; **213**: 1555-1570 [PMID: 27432944 DOI: 10.1084/jem.20151932]

10 **Xiao Y**, Tang J, Guo H, Zhao Y, Tang R, Ouyang S, Zeng Q, Rappleye CA, Rajaram MV, Schlesinger LS, Tao L, Brown GD, Langdon WY, Li BT, Zhang J. Targeting CBLB as a potential therapeutic approach for disseminated candidiasis. *Nat Med* 2016; **22**: 906-914 [PMID: 27428899 DOI: 10.1038/nm.4141]

11 **Pickart CM**. Mechanisms underlying ubiquitination. *Annu Rev Biochem* 2001; **70**: 503-533 [PMID: 11395416 DOI: 10.1146/annurev.biochem.70.1.503]

12 **Thien CB**, Langdon WY. Cbl: many adaptations to regulate protein tyrosine kinases. *Nat Rev Mol Cell Biol* 2001; **2**: 294-307 [PMID: 11283727 DOI: 10.1038/35067100]

13 **Ettenberg SA,** Keane MM, Nau MM, Frankel M, Wang LM, Pierce JH, Lipkowitz S. cbl-b inhibits epidermal growth factor receptor signaling. *Oncogene* 1999; **18**: 1855-1866 [PMID: 10086340 DOI: 10.1038/sj.onc.1202499]

14 **Sohn HW**, Gu H, Pierce SK. Cbl-b negatively regulates B cell antigen receptor signaling in mature B cells through ubiquitination of the tyrosine kinase Syk. *J Exp Med* 2003; **197**: 1511-1524 [PMID: 12771181 DOI: 10.1084/jem.20021686]

15 **Meng W**, Sawasdikosol S, Burakoff SJ, Eck MJ. Structure of the amino-terminal domain of Cbl complexed to its binding site on ZAP-70 kinase. *Nature* 1999; **398**: 84-90 [PMID: 10078535 DOI: 10.1038/18050]

16 **Levkowitz G**, Waterman H, Ettenberg SA, Katz M, Tsygankov AY, Alroy I, Lavi S, Iwai K, Reiss Y, Ciechanover A, Lipkowitz S, Yarden Y. Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. *Mol Cell* 1999; **4**: 1029-1040 [PMID: 10635327 DOI: 10.1016/S1097-2765(00)80231-2]

17 **Elly C**, Witte S, Zhang Z, Rosnet O, Lipkowitz S, Altman A, Liu YC. Tyrosine phosphorylation and complex formation of Cbl-b upon T cell receptor stimulation. *Oncogene* 1999; **18**: 1147-1156 [PMID: 10022120 DOI: 10.1038/sj.onc.1202411]

18 **Kobashigawa Y**, Tomitaka A, Kumeta H, Noda NN, Yamaguchi M, Inagaki F. Autoinhibition and phosphorylation-induced activation mechanisms of human cancer and autoimmune disease-related E3 protein Cbl-b. *Proc Natl Acad Sci USA* 2011; **108**: 20579-20584 [PMID: 22158902 DOI: 10.1073/pnas.1110712108]

19 **Yokoi N**, Komeda K, Wang HY, Yano H, Kitada K, Saitoh Y, Seino Y, Yasuda K, Serikawa T, Seino S. Cblb is a major susceptibility gene for rat type 1 diabetes mellitus. *Nat Genet* 2002; **31**: 391-394 [PMID: 12118252 DOI: 10.1038/ng927]

20 **Yokoi N**, Fujiwara Y, Wang HY, Kitao M, Hayashi C, Someya T, Kanamori M, Oiso Y, Tajima N, Yamada Y, Seino Y, Ikegami H, Seino S. Identification and functional analysis of CBLB mutations in type 1 diabetes. *Biochem Biophys Res Commun* 2008; **368**: 37-42 [PMID: 18201552 DOI: 10.1016/j.bbrc.2008.01.032]

21 **Sanna S**, Pitzalis M, Zoledziewska M, Zara I, Sidore C, Murru R, Whalen MB, Busonero F, Maschio A, Costa G, Melis MC, Deidda F, Poddie F, Morelli L, Farina G, Li Y, Dei M, Lai S, Mulas A, Cuccuru G, Porcu E, Liang L, Zavattari P, Moi L, Deriu E, Urru MF, Bajorek M, Satta MA, Cocco E, Ferrigno P, Sotgiu S, Pugliatti M, Traccis S, Angius A, Melis M, Rosati G, Abecasis GR, Uda M, Marrosu MG, Schlessinger D, Cucca F. Variants within the immunoregulatory CBLB gene are associated with multiple sclerosis. *Nat Genet* 2010; **42**: 495-497 [PMID: 20453840 DOI: 10.1038/ng.584]

22 **Yokoi N**, Hayashi C, Fujiwara Y, Wang HY, Seino S. Genetic reconstitution of autoimmune type 1 diabetes with two major

susceptibility genes in the rat. *Diabetes* 2007; **56**: 506-512 [PMID: 17259398 DOI: 10.2337/db06-1027]
23 **Weisberg SP**, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003; **112**: 1796-1808 [PMID: 14679176 DOI: 10.1172/JCI19246]
24 **Yamamoto M**, Akira S. Lipid A receptor TLR4-mediated signaling pathways. *Adv Exp Med Biol* 2010; **667**: 59-68 [PMID: 20665200 DOI: 10.1007/978-1-4419-1603-7_6]
25 **Shi H**, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* 2006; **116**: 3015-3025 [PMID: 17053832 DOI: 10.1172/JCI28898]
26 **Suganami T**, Tanimoto-Koyama K, Nishida J, Itoh M, Yuan X, Mizuarai S, Kotani H, Yamaoka S, Miyake K, Aoe S, Kamei Y, Ogawa Y. Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. *Arterioscler Thromb Vasc Biol* 2007; **27**: 84-91 [PMID: 17082484 DOI: 10.1161/01.ATV.0000251608.09329.9a]
27 **Saberi M**, Woods NB, de Luca C, Schenk S, Lu JC, Bandyopadhyay G, Verma IM, Olefsky JM. Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. *Cell Metab* 2009; **10**: 419-429 [PMID: 19883619 DOI: 10.1016/j.cmet.2009.09.006]
28 **Schaeffler A**, Gross P, Buettner R, Bollheimer C, Buechler C, Neumeier M, Kopp A, Schoelmerich J, Falk W. Fatty acid-induced induction of Toll-like receptor-4/nuclear factor-kappaB pathway in adipocytes links nutritional signalling with innate immunity. *Immunology* 2009; **126**: 233-245 [PMID: 18624726 DOI: 10.1111/j.1365-2567.2008.02892.x]
29 **Pal D**, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, Ray S, Majumdar SS, Bhattacharya S. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. *Nat Med* 2012; **18**: 1279-1285 [PMID: 22842477 DOI: 10.1038/nm.2851]
30 **Ochi A**, Mori K, Emoto M, Nakatani S, Morioka T, Motoyama K, Fukumoto S, Imanishi Y, Koyama H, Ishimura E, Inaba M. Direct inhibitory effects of pioglitazone on hepatic fetuin-A expression. *PLoS One* 2014; **9**: e88704 [PMID: 24551137 DOI: 10.1371/journal.pone.0088704]
31 **Nguyen MT**, Favelyukis S, Nguyen AK, Reichart D, Scott PA, Jenn A, Liu-Bryan R, Glass CK, Neels JG, Olefsky JM. A subpopulation of macrophages infiltrates hypertrophic adipose tissue and is activated by free fatty acids via Toll-like receptors 2 and 4 and JNK-dependent pathways. *J Biol Chem* 2007; **282**: 35279-35292 [PMID: 17916553 DOI: 10.1074/jbc.M706762200]
32 **Arkan MC**, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M. IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* 2005; **11**: 191-198 [PMID: 15685170 DOI: 10.1038/nm1185]
33 **Han MS**, Jung DY, Morel C, Lakhani SA, Kim JK, Flavell RA, Davis RJ. JNK expression by macrophages promotes obesity-induced insulin resistance and inflammation. *Science* 2013; **339**: 218-222 [PMID: 23223452 DOI: 10.1126/science.1227568]
34 **Solinas G**, Vilcu C, Neels JG, Bandyopadhyay GK, Luo JL, Naugler W, Grivennikov S, Wynshaw-Boris A, Scadeng M, Olefsky JM, Karin M. JNK1 in hematopoietically derived cells contributes to diet-induced inflammation and insulin resistance without affecting obesity. *Cell Metab* 2007; **6**: 386-397 [PMID: 17983584 DOI: 10.1016/j.cmet.2007.09.011]
35 **Hirasaka K**, Kohno S, Goto J, Furochi H, Mawatari K, Harada N, Hosaka T, Nakaya Y, Ishidoh K, Obata T, Ebina Y, Gu H, Takeda S, Kishi K, Nikawa T. Deficiency of Cbl-b gene enhances infiltration and activation of macrophages in adipose tissue and causes peripheral insulin resistance in mice. *Diabetes* 2007; **56**: 2511-2522 [PMID: 17601987 DOI: 10.2337/db06-1768]
36 **Abe T**, Hirasaka K, Kagawa S, Kohno S, Ochi A, Utsunomiya K, Sakai A, Ohno A, Teshima-Kondo S, Okumura Y, Oarada M, Maekawa Y, Terao J, Mills EM, Nikawa T. Cbl-b is a critical regulator of macrophage activation associated with obesity-induced insulin resistance in mice. *Diabetes* 2013; **62**: 1957-1969 [PMID: 23349502 DOI: 10.2337/db12-0677]
37 **Proudfoot AE**. Chemokine receptors: multifaceted therapeutic targets. *Nat Rev Immunol* 2002; **2**: 106-115 [PMID: 11910892 DOI: 10.1038/nri722]
38 **Weisberg SP**, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, Charo I, Leibel RL, Ferrante AW. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *J Clin Invest* 2006; **116**: 115-124 [PMID: 16341265 DOI: 10.1172/JCI24335]
39 **Nio Y**, Yamauchi T, Iwabu M, Okada-Iwabu M, Funata M, Yamaguchi M, Ueki K, Kadowaki T. Monocyte chemoattractant protein-1 (MCP-1) deficiency enhances alternatively activated M2 macrophages and ameliorates insulin resistance and fatty liver in lipoatrophic diabetic A-ZIP transgenic mice. *Diabetologia* 2012; **55**: 3350-3358 [PMID: 22983634 DOI: 10.1007/s00125-012-2710-2]
40 **Sullivan TJ**, Miao Z, Zhao BN, Ertl LS, Wang Y, Krasinski A, Walters MJ, Powers JP, Dairaghi DJ, Baumgart T, Seitz LC, Berahovich RD, Schall TJ, Jaen JC. Experimental evidence for the use of CCR2 antagonists in the treatment of type 2 diabetes. *Metabolism* 2013; **62**: 1623-1632 [PMID: 23953944 DOI: 10.1016/j.metabol.2013.06.008]
41 **Bolus WR**, Gutierrez DA, Kennedy AJ, Anderson-Baucum EK, Hasty AH. CCR2 deficiency leads to increased eosinophils, alternative macrophage activation, and type 2 cytokine expression in adipose tissue. *J Leukoc Biol* 2015; **98**: 467-477 [PMID: 25934927 DOI: 10.1189/jlb.3HI0115-018R]
42 **Obstfeld AE**, Sugaru E, Thearle M, Francisco AM, Gayet C, Ginsberg HN, Ables EV, Ferrante AW. C-C chemokine receptor 2 (CCR2) regulates the hepatic recruitment of myeloid cells that promote obesity-induced hepatic steatosis. *Diabetes* 2010; **59**: 916-925 [PMID: 20103702 DOI: 10.2337/db09-1403]
43 **Tamura Y**, Sugimoto M, Murayama T, Minami M, Nishikaze Y, Ariyasu H, Akamizu T, Kita T, Yokode M, Arai H. C-C chemokine receptor 2 inhibitor improves diet-induced development of insulin resistance and hepatic steatosis in mice. *J Atheroscler Thromb* 2010; **17**: 219-228 [PMID: 20179360 DOI: 10.5551/jat.3368]
44 **Wells CM**, Bhavsar PJ, Evans IR, Vigorito E, Turner M, Tybulewicz V, Ridley AJ. Vav1 and Vav2 play different roles in macrophage migration and cytoskeletal organization. *Exp Cell Res* 2005; **310**: 303-310 [PMID: 16137676 DOI: 10.1016/j.yexcr.2005.07.015]
45 **Deckert M**, Tartare-Deckert S, Couture C, Mustelin T, Altman A. Functional and physical interactions of Syk family kinases with the Vav proto-oncogene product. *Immunity* 1996; **5**: 591-604 [PMID: 8986718 DOI: 10.1016/S1074-7613(00)80273-3]
46 **Tong H**, Zhao B, Shi H, Ba X, Wang X, Jiang Y, Zeng X. c-Abl tyrosine kinase plays a critical role in β2 integrin-dependent neutrophil migration by regulating Vav1 activity. *J Leukoc Biol* 2013; **93**: 611-622 [PMID: 23325923 DOI: 10.1189/jlb.1012487]
47 **Naramura M**, Jang IK, Kole H, Huang F, Haines D, Gu H. c-Cbl and Cbl-b regulate T cell responsiveness by promoting ligand-induced TCR down-modulation. *Nat Immunol* 2002; **3**: 1192-1199 [PMID: 12415267 DOI: 10.1038/ni855]
48 **Krawczyk CM**, Jones RG, Atfield A, Bachmaier K, Arya S, Odermatt B, Ohashi PS, Penninger JM. Differential control of CD28-regulated in vivo immunity by the E3 ligase Cbl-b. *J Immunol* 2005; **174**: 1472-1478 [PMID: 15661906 DOI: 10.4049/jimmunol.174.3.1472]
49 **Chuang TH**, Ulevitch RJ. Triad3A, an E3 ubiquitin-protein ligase regulating Toll-like receptors. *Nat Immunol* 2004; **5**: 495-502 [PMID: 15107846 DOI: 10.1038/ni1066]
50 **Mansell A**, Smith R, Doyle SL, Gray P, Fenner JE, Crack PJ, Nicholson SE, Hilton DJ, O'Neill LA, Hertzog PJ. Suppressor of cytokine signaling 1 negatively regulates Toll-like receptor signaling by mediating Mal degradation. *Nat Immunol* 2006; **7**: 148-155 [PMID: 16415872 DOI: 10.1038/ni1299]
51 **Zadjali F**, Santana-Farre R, Vesterlund M, Carow B, Mirecki-Garrido M, Hernandez-Hernandez I, Flodström-Tullberg M, Parini P, Rottenberg M, Norstedt G, Fernandez-Perez L, Flores-Morales A. SOCS2 deletion protects against hepatic steatosis but worsens insulin resistance in high-fat-diet-fed mice. *FASEB J* 2012; **26**: 3282-3291 [PMID: 22562833 DOI: 10.1096/fj.12-205583]
52 **Hu MM**, Xie XQ, Yang Q, Liao CY, Ye W, Lin H, Shu HB. TRIM38 Negatively Regulates TLR3/4-Mediated Innate Immune and

Inflammatory Responses by Two Sequential and Distinct Mechanisms. *J Immunol* 2015; **195**: 4415-4425 [PMID: 26392463 DOI: 10.4049/jimmunol.1500859]

53 **Bachmaier K**, Toya S, Gao X, Triantafillou T, Garrean S, Park GY, Frey RS, Vogel S, Minshall R, Christman JW, Tiruppathi C, Malik AB. E3 ubiquitin ligase Cblb regulates the acute inflammatory response underlying lung injury. *Nat Med* 2007; **13**: 920-926 [PMID: 17618294 DOI: 10.1038/nm1607]

54 **Fessler MB**, Rudel LL, Brown JM. Toll-like receptor signaling links dietary fatty acids to the metabolic syndrome. *Curr Opin Lipidol* 2009; **20**: 379-385 [PMID: 19625959 DOI: 10.1097/MOL.0b013e32832fa5c4]

55 **Medvedev AE**, Piao W, Shoenfelt J, Rhee SH, Chen H, Basu S, Wahl LM, Fenton MJ, Vogel SN. Role of TLR4 tyrosine phosphorylation in signal transduction and endotoxin tolerance. *J Biol Chem* 2007; **282**: 16042-16053 [PMID: 17392283 DOI: 10.1074/jbc.M606781200]

56 **Miller YI**, Choi SH, Wiesner P, Bae YS. The SYK side of TLR4: signalling mechanisms in response to LPS and minimally oxidized LDL. *Br J Pharmacol* 2012; **167**: 990-999 [PMID: 22776094 DOI: 10.1111/j.1476-5381.2012.02097.x]

57 **Lu Z**, Zhang X, Li Y, Lopes-Virella MF, Huang Y. TLR4 antagonist attenuates atherogenesis in LDL receptor-deficient mice with diet-induced type 2 diabetes. *Immunobiology* 2015; **220**: 1246-1254 [PMID: 26162692 DOI: 10.1016/j.imbio.2015.06.016]

58 **Kim HM**, Park BS, Kim JI, Kim SE, Lee J, Oh SC, Enkhbayar P, Matsushima N, Lee H, Yoo OJ, Lee JO. Crystal structure of the TLR4-MD-2 complex with bound endotoxin antagonist Eritoran. *Cell* 2007; **130**: 906-917 [PMID: 17803912 DOI: 10.1016/j.cell.2007.08.002]

59 **Ciccone MM,** Scicchitano P, Cameli M, Cecere A, Cortese F, Dentamaro I, Gentile F, Gesuaido M, Maiello M, Modesti PA, Muiesan ML, Novo S, Palmiero P, Saba PS, Zito A, Mattioli AV, Pedrinelli R. Endothelial Function in Pre-diabetes, Diabetes and Diabetic Cardiomyopathy: A Review. *J Diabetes Metab* 2014; **5**: 364 [DOI: 10.4172/2155-6156.1000364]

60 **Festa A**, Hanley AJ, Tracy RP, D'Agostino R, Haffner SM. Inflammation in the prediabetic state is related to increased insulin resistance rather than decreased insulin secretion. *Circulation* 2003; **108**: 1822-1830 [PMID: 14517163 DOI: 10.1161/01.CIR.0000091339.70120.53]

61 **Nakao R**, Hirasaka K, Goto J, Ishidoh K, Yamada C, Ohno A, Okumura Y, Nonaka I, Yasutomo K, Baldwin KM, Kominami E, Higashibata A, Nagano K, Tanaka K, Yasui N, Mills EM, Takeda S, Nikawa T. Ubiquitin ligase Cbl-b is a negative regulator for insulin-like growth factor 1 signaling during muscle atrophy caused by unloading. *Mol Cell Biol* 2009; **29**: 4798-4811 [PMID: 19546233 DOI: 10.1128/MCB.01347-08]

P- Reviewer: Al-Gayyar MMH, Ciccone MM, Guzman-Gutierrez E, Hekmatdoost A **S- Editor**: Song XX **L- Editor**: A **E- Editor**: Lu YJ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/ *World J Diabetes* 2017 March 15; 8(3): 104-111

DOI: 10.4239/wjd.v8.i3.104 ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Statin, testosterone and phosphodiesterase 5-inhibitor treatments and age related mortality in diabetes

Geoffrey Hackett, Peter W Jones, Richard C Strange, Sudarshan Ramachandran

Geoffrey Hackett, Department of Urology, University of Bedfordshire, Bedfordshire, Luton LU1 3JU, United Kingdom

Geoffrey Hackett, Department of Urology, Heart of England NHS Foundation Trust, West Midlands B75 7RR, United Kingdom

Peter W Jones, Richard C Strange, Metabolic Research Group, Institute of Science and Technology in Medicine, Keele University, Keele ST5 5BG, United Kingdom

Sudarshan Ramachandran, Department of Clinical Biochemistry, Heart of England NHS Foundation Trust, West Midlands B75 7RR, United Kingdom

Sudarshan Ramachandran, Department of Clinical Biochemistry, University Hospitals of North Midlands/Faculty of Health Sciences, Staffordshire University, Stoke-on-Trent ST4 2DE, United Kingdom

Author contributions: The original study was designed by Hackett G; Strange RC and Ramachandran S have had an interest in the work of Gompertz B and conceived the idea of investigating age related mortality patterns in different cohorts and studying how interventions alter age related mortality; all authors contributed to the data analysis this work being supervised by Jones PW as well as drafting, revising and approving the final version of the manuscript.

Supported by Bayer plc to University of Bedfordshire (ref: SOP ID: BSP-SOP-040); Bayer plc played no part in the design, conduct of the study, data collection, statistical analyses or preparation of the manuscript.

Institutional review board statement: The study was reviewed and approved by the following bodies: National Research Ethics Service (West Midlands Ethics Service) 08/H1208/30; National Institute for Health Research (Birmingham and the Black Country Comprehensive Local Research Network), RM and G reference Number 1268; Warwickshire Primary Care Trust (West Midlands (South) Comprehensive Local Research Network), reference WAR230909; Institute of Diabetes for Older People, Beds and Herts Postgraduate Medical School, University of Bedfordshire (ref: SOP ID: BSP-SOP-040); Bayer plc (ref: SOP ID: BSP-SOP-040): funding organisation; Institute for Science and Technology in Medicine, Keele University Medical School: approval for publication (Professor N Forsyth, Director of the Institute).

Informed consent statement: All participants in the intervention study (BLAST) provided informed written consent prior to study enrolment. The long term follow-up of the screened individuals receiving standard care was approved as an audit by Primary Care Trusts.

Conflict-of-interest statement: No potential conflicts of interest relevant to this article were reported.

Data sharing statement: Technical appendix, statistical code, and dataset are available from the corresponding author at (sud.ramachandran@heartofengland.nhs.uk). Participants gave informed consent for anonymous data to be shared with health authorities and published in scientific journals.

Manuscript source: Invited manuscript

Correspondence to: Sudarshan Ramachandran, PhD, FRC Path, Professor, Department of Clinical Biochemistry, Heart of England NHS Foundation Trust, Rectory Road, Sutton Coldfield, West Midlands B75 7RR, United Kingdom. sud.ramachandran@heartofengland.nhs.uk
Telephone: +44-121-4247246
Fax: +44-121-3111800

Received: October 27, 2016
Peer-review started: October 31, 2016
First decision: December 1, 2016
Revised: December 14, 2016
Accepted: January 2, 2017
Article in press: January 3, 2017
Published online: March 15, 2017

Abstract

AIM

To determine how statins, testosterone (T) replacement therapy (TRT) and phosphodiesterase 5-inhibitors (PDE5I) influence age related mortality in diabetic men.

METHODS

We studied 857 diabetic men screened for the BLAST study, stratifying them (mean follow-up = 3.8 years) into: (1) Normal T levels/untreated (total T > 12 nmol/L and free T > 0.25 nmol/L), Low T/untreated and Low T/treated; (2) PDE5I/untreated and PDE5I/treated; and (3) statin/untreated and statin/treated groups. The relationship between age and mortality, alone and with T/TRT, statin and PDE5I treatment was studied using logistic regression. Mortality probability and 95%CI were calculated from the above models for each individual.

RESULTS

Age was associated with mortality (logistic regression, OR = 1.10, 95%CI: 1.08-1.13, $P < 0.001$). With all factors included, age (OR = 1.08, 95%CI: 1.06-1.11, $P < 0.001$), Low T/treated (OR = 0.38, 95%CI: 0.15-0.92, $P = 0.033$), PDE5I/treated (OR = 0.17, 95%CI: 0.053-0.56, $P = 0.004$) and statin/treated (OR = 0.59, 95%CI: 0.36-0.97, $P = 0.038$) were associated with lower mortality. Age related mortality was as described by Gompertz, $r^2 = 0.881$ when Ln (mortality) was plotted against age. The probability of mortality and 95%CI (from logistic regression) of individuals, treated/untreated with the drugs, alone and in combination was plotted against age. Overlap of 95%CI lines was evident with statins and TRT. No overlap was evident with PDE5I alone and with statins and TRT, this suggesting a change in the relationship between age and mortality.

CONCLUSION

We show that statins, PDE5I and TRT reduce mortality in diabetes. PDE5I, alone and with the other treatments significantly alter age related mortality in diabetic men.

Key words: Type 2 diabetes; Mortality; Gompertz-Makeham equation; Phosphodiesterase 5 inhibitors; Male hypogonadism; Statins; Testosterone replacement therapy

Core tip: We have described a study of men with type 2 diabetes showing that mortality rates are in accordance with the pattern described nearly 200 years ago by Benjamin Gompertz. The data show that statin, phosphodiesterase 5 inhibitors (PDE5I) and testosterone replacement in hypogonadal men reduce all-cause mortality. PDE5I, alone and in combination with the other 2 agents alters the association between age and mortality, thus improving prognosis. The graphical illustrations adopted in this paper communicate the impact of medical intervention very effectively to patients and this could potentially improve compliance leading to significant clinical benefit.

Hackett G, Jones PW, Strange RC, Ramachandran S. Statin, testosterone and phosphodiesterase 5-inhibitor treatments and age related mortality in diabetes. *World J Diabetes* 2017; 8(3): 104-111 Available from: URL: http://www.wjgnet.com/1948-9358/full/v8/i3/104.htm DOI: http://dx.doi.org/10.4239/wjd.v8.i3.104

INTRODUCTION

In 1825, Benjamin Gompertz described a law defining human mortality based on the equation, $\mu(x) = \alpha \times e^{\beta x}$, with $\mu(x)$ being the mortality rate at age x years and, α and β (considered to be the ageing rate) being constants[1]. Subsequently, Makeham proposed a modified Gompertz equation, $\mu(x) = \alpha \times e^{\beta x} + y$, that included a factor (y) describing extrinsic mortality thereby allowing the effect of age -independent and -dependent factors on mortality to be studied[1,2]. Avoidance of external factors such as conflict or starvation results in the age-independent component having less impact on death rate and therefore, mortality increases exponentially with age. It has been suggested that β is similar for all populations and it is the intercept (α) that varies[3]. In developed countries, the age-independent intercept is influenced by medical care as many treatments have a significant impact on mortality though factors encountered in early life can also influence later outcomes[4].

Mortality in type 2 diabetes (T2DM) is associated with age and therapy. Patients have a 1.5-2.5 fold higher mortality than the general population[5,6] with men and women suffering a mean 7.5 years and 7 years reduction in life expectancy respectively[7]. This increase in mortality appears related to the age at diagnosis, being lower in patients diagnosed their late 70 s compared with those aged in their mid-40 s[8].

Several inter-related pathologies are associated with mortality in T2DM and accordingly, statins and in men, testosterone replacement therapy (TRT) and phosphodiesterase 5 inhibitors (PDE5I) are commonly prescribed. Since rates of cardiovascular disease (CVD) associated mortality are 2-4 times higher than in non-diabetics, reduction of serum LDL-cholesterol is a key aim[9,10]. Statins are the mainstay of lipid lowering therapy with trials demonstrating marked reductions in CVD in T2DM patients[9,11]. Male hypogonadism characterised by low serum testosterone and sexual dysfunction is common in diabetics and also linked with mortality[12-15]. Studies in men with diabetes show low serum free testosterone is associated with carotid atherosclerosis identified by carotid artery intimal thickness and plaque score[16]. All-cause mortality is greater in those with low compared with normal total testosterone levels[17]. Three studies, two in men with T2DM, suggest TRT reduces all-cause mortality[17-19]. A randomized controlled study of men with T2DM showed TRT improves symptoms

of hypogonadism, the different symptoms significantly improving at varying testosterone thresholds[20]. Importantly, in T2DM patients, erectile dysfunction (ED) appears to be an independent predictor of vascular disease and mortality[13-15]. PDE5I have beneficial effects on endothelial function, insulin resistance and possess potentially cardio-protective properties[15,21,22]. Gazzaruso *et al*[15] showed that PDE5I use in T2DM men was associated with reductions in major adverse cardiac events and angiography confirmed coronary artery disease. Our longitudinal prospective study indicated that PDE5I use was possibly associated with reduced mortality independent of TRT and statin therapy[19]. Further, Anderson *et al*[23] reported a 31% reduction in all-cause mortality and 26% reduction in myocardial infarction in men with T2DM prescribed PDE5I.

We recently reported in a prospective longitudinal study of 857 men with T2DM that TRT and PDE5I were independently associated with increased survival in men with T2DM[19]. Importantly baseline body weight, body mass index, lipids, glycaemic control and hypertension were not associated with mortality. We now describe a retrospective analysis of this cohort determining the association between age and mortality, establishing whether the mortality rate follows the pattern described by Gompertz and estimating how testosterone status and treatments (statins, TRT and PDE5I) alter this relationship.

MATERIALS AND METHODS

This study is a retrospective analysis of follow-up data obtained in 857 men with T2DM screened following informed consent for the randomised double blind placebo controlled BLAST (Birmingham, Lichfield, Atherstone, Sutton and Tamworth) study designed to investigate the effects of long acting testosterone on clinical symptoms and metabolic parameters over a 30 wk treatment period[24]. The patients were listed in the registers of 5 English Midlands practices and initially screened for total testosterone (TT) and free testosterone (FT) during April 2007-April 2009. Based on total and free testosterone levels, the 857 men were classified as Normal T (total testosterone > 12 nmol/L and free testosterone > 0.25 nmol/L) or Low T (total testosterone $\leqslant$ 12 nmol/L or free testosterone $\leqslant$ 0.25 nmol/L) with a second measurement taken at least 2 wk later in men with TT $\leqslant$ 12 nmol/L, according to the Endocrine Society clinical practice guidelines[25]. The BLAST intervention study were approved by the West Midlands Regional Ethics Committee (reference: 08/H1208/30), the National Institute for Health Research (Birmingham and the Black Country Comprehensive Local Research Park - RM&G reference: 1268) and Warwickshire Primary Care Trust (reference: WAR230909) with the long term follow-up approved as an audit by all the appropriate Primary Care Trust Ethics Committees.

United Kingdom Primary care diabetes care treatment of glycaemic control, dyslipidaemia and hypertension is protocol and guideline driven as part of the Quality Outcomes Framework initiative. TRT was prescribed according to the BLAST study programme[24,26]. The United Kingdom NHS regulations allowed PDE5I prescribing for ED in men with diabetes with a suggested regime of 1 dose/wk[27].

The 857 men were categorised by statin, TRT and PDE5I treatment at death or final visit, firstly, by statin treatment; 195 men were Statin/untreated and 662 men Statin/treated, secondly by hypogonadism and TRT; 320 men were Normal T (TT > 12 nmol/L and FT > 0.25 nmol/L)/untreated, 362 men were Low T (TT $\leqslant$ 12 nmol/L or FT $\leqslant$ 0.25 nmol/L)/untreated and 175 men were Low T/treated (TT $\leqslant$ 12 nmol/L or FT $\leqslant$ 0.25 nmol/L)/treated and thirdly, by PDE5I treatment; 682 men were PDE5I/untreated and 175 men were PDE5I/treated. Mortality data was collected from the general practice databases, hospital letters and death certificates.

Laboratory methods

Statin and TRT prescribing was based on protocols based on laboratory measurements. Serum sex hormone binding globulin, albumin and lipids were analysed using a Roche Modular automated analyzer (Roche Diagnostics, Burgess Hill, United Kingdom). Early morning fasting TT was measured using the validated Roche common platform immunoassay. FT was calculated using the Vermeulen *et al*[28].

Stata version 8 (College Station, TX) was used for statistical analyses with all-cause mortality as primary end point. Differences in mortality between the alive and deceased groups were identified using χ^2 (statin, PDE5I, TRT and hypogonadism) and unpaired *t*-test (age at death or final visit). Logistic (and logit) regression was initially carried out to study the association between death/survival (dichotomous outcome) and age at death or final follow-up visit as the independent variable. Subsequently, separate models were developed with each treatment (statins, testosterone (status and treatment) and PDE5I) being included with age at death or final visit as the independent variables. Discrete ordinal variables were factorised with one category selected as reference. In the testosterone groups, Low T/untreated was selected as reference as Normal T/untreated and Low T/treated differed by one characteristic, *i.e.,* TT and FT concentration and TRT respectively. Patients on statins and PDE5I were compared to those not on treatment (reference groups). We estimated individual probability of mortality (and 95%CI for each man using separate logistic regression models. The statistical approaches used were reviewed by author, Professor Peter W Jones, Professor of Medical Statistics, Keele University, and considered appropriate.

RESULTS

Table 1 presents the data used to compare the age and treatment details of men who either survived or died during the study. The Table shows the mean age of the total study group and of the 754 men alive at study end

Table 1 Mortality in men with type 2 diabetes stratified by treatment with statins, testosterone status/treatment, phosphodiesterase 5-inhibitors and combinations of treatments *n* (%)

	Total group	Alive	Deceased	*P* value
Patient *n*	857	754	103	
Mean age at death or last visit/SD (yr)	67.4/11.6	66.2/11.3	76.2/10.2	< 0.0001[1]
Patient numbers (%) stratified by treatment				
Statin/untreated	195 (22.8)	162 (21.5)	33 (32.0)	0.017[2]
Statin/treated	662 (77.3)	592 (78.5)	70 (68.0)	
Normal T/untreated	320 (37.3)	284 (37.7)	36 (35.0)	< 0.001[2]
Low T/untreated	362 (42.2)	301 (39.9)	61 (59.2)	
Low T/treated	175 (20.4)	169 (22.4)	6 (5.8)	
PDE5I/untreated	682 (79.6)	582 (77.2)	100 (97.1)	< 0.001[2]
PDE5I/treated	175 (20.4)	172 (22.8)	3 (2.9)	
Not on any of the above therapeutic agents	125 (14.6)	92 (12.2)	33 (32.0)	0.002[2]
On all 3 therapeutic agents	45 (5.3)	43 (5.7)	2 (1.9)	

[1]Unpaired *t* test; [2]χ^2 analysis.

Table 2 Association between age and mortality corrected for statin treatment, testosterone status/treatment and phosphodiesterase 5-inhibitors treatment

	OR (95%CI)	*P* value
Model a		
Age (yr)	1.10 (1.08-1.13)	< 0.001
Model b		
Age (yr)	1.10 (1.07-1.13)	< 0.001
Statin/untreated	Reference	
Statin/treated	0.63 (0.39-1.01)	0.057
Model c		
Age (yr)	1.10 (1.07-1.12)	< 0.001
Normal T/untreated	0.61 (0.42-1.07)	0.092
Low T/untreated	Reference	
Low T/treated	0.31 (0.13-0.75)	0.009
Model d		
Age (yr)	1.09 (1.07-1.12)	< 0.001
PDE5I/untreated	Reference	
PDE5I/treated	0.16 (0.051-0.54)	0.003
Model e		
Age (yr)	1.08 (1.06-1.11)	< 0.001
Statin/untreated	Reference	
Statin/treated	0.59 (0.36-0.97)	0.038
Normal T/untreated	0.69 (0.43-1.10)	0.120
Low T/untreated	Reference	
Low T/treated	0.38 (0.15-0.92)	0.033
PDE5I/untreated	Reference	
PDE5I/treated	0.17 (0.053-0.56)	0.004

PDE5I: Phosphodiesterase 5-inhibitors.

and 103 deceased men. Mean age in the deceased group was significantly higher ($P < 0.0001$) than in survivors. Table 1 also shows the proportion of alive/deceased men treated with statin or PDE5I. In the deceased group, a significantly lower proportion of men were treated with statins (68.0%, $P = 0.017$) or PDE5I (2.9%, $P < 0.001$) compared with survivors (78.5%, 22.8% respectively). To assess the impact of hypogonadism and TRT on mortality, we stratified the 857 men into three groups; Normal T/untreated (eugonadal), Low T/untreated and Low T/treated. Table 1 shows in the deceased group that the proportions of men given TRT (5.8%, $P < 0.001$) or who were eugonadal (35.0%, $P = 0.037$) was significantly lower than that of men in the Low T/untreated group (59.2%).

Importantly, in two of the treatment groups the age at final visit of survivors varied; PDE5I treatment (PDE5I/untreated: Mean age = 67.2 ± 10.1 years, PDE5I/treated: Mean age = 62.7 ± 10.0 years, $P < 0.0001$) and TRT (Low T/untreated: 67.3 ± 11.3 years, Low T/treated: 61.8 ± 10.9 years, $P < 0.0001$) patients. No corresponding difference in age at final visit in survivors was observed in the Statin/untreated *vs* Statin/treated and Normal T/untreated *vs* Low T/untreated groups. Age at death did not significantly differ with statin (Statin/untreated: Mean age = 77.0 ± 10.5 years, Statin/treated: Mean age = 75.8 ± 10.1 years, $P = 0.56$) or PDE5I treatment (PDE5I/untreated: Mean age = 76.4 ± 10.1 years, PDE5I/treated: Mean age = 67.0 ± 13.3 years, $P = 0.11$). Importantly, only 3 patients on PDE5I treatment died during follow-up (Table 1). Interestingly, age at death varied between the testosterone groups (Normal T/untreated: Mean age = 73.9 ± 10.6 years *vs* Low T/untreated: Mean age = 78.4 ± 8.9 years, $P = 0.0.028$, Low T/untreated: Mean age = 78.4 ± 8.9 *vs* Low T/treated: Mean age 66.3 ± 13.1 years, $P = 0.0034$).

As age at death or final visit differed between the treatment and testosterone status groups we used logistic regression analyses to see if the associations in Table 1 were independent. Table 2 shows age is associated with mortality regardless of the other factors added to regression models (Models a-e). Significant reduction in mortality was observed with TRT (Low T men - Model c) and PDE5I (Model d) treatments while the benefit due to statins approached significance (Model b). All 3 treatments were significantly associated with decreased mortality when entered together (Model e).

We determined if our mortality data adhered to the Gompertz-Makeham equation. Figure 1 shows mortality rate (as % and logarithmic values) plotted against age (as 5 year categories). A linear relationship was observed between Ln (mortality) and age ($R^2 = 0.881$) suggesting that the mortality observed in our cohort did fit the pattern initially described by Gompertz.

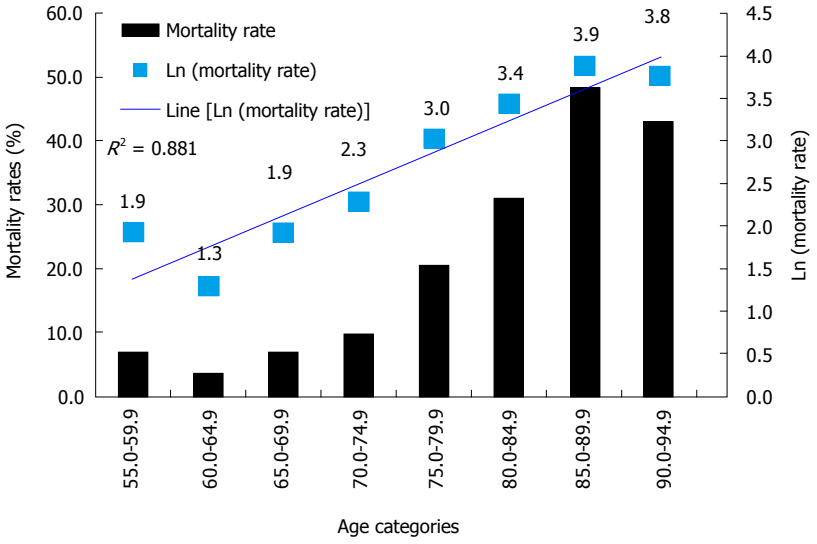
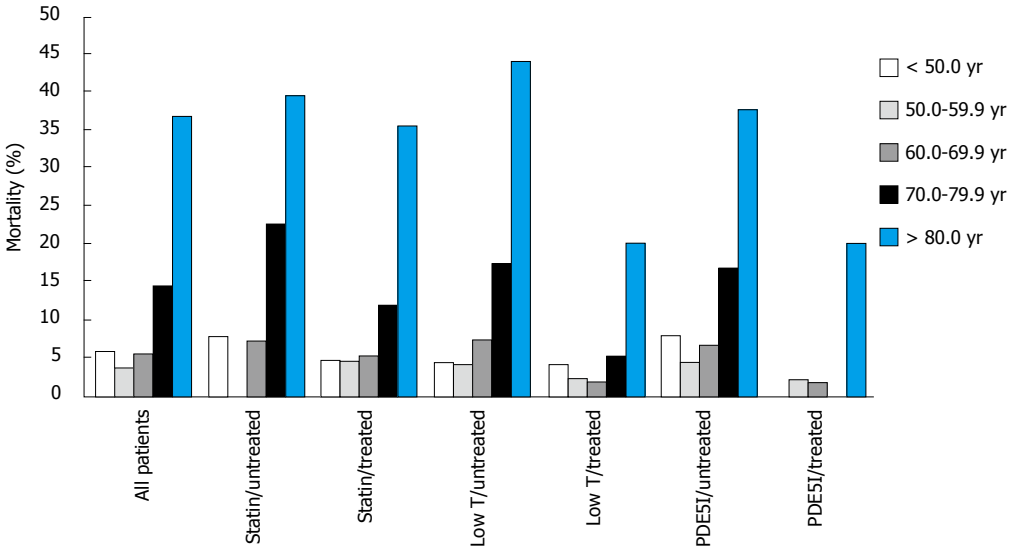


Figure 1 Association between Ln (mortality rate) and age.



Age (yr)	All patients	Mortality %	Statin/ untreated	Mortality %	Statin/ treated	Mortality %	Low T/ untreated	Mortality %	Low T/treated	Mortality %	PDE5I/ untreated	Mortality %	PDE5I/ treated	Mortality %
< 50.0	70	5.7	26	7.7	44	4.6	24	4.2	25	4.0	51	7.8	19	0.0
50.0-59.9	143	3.5	28	0.0	115	4.4	50	4.0	48	2.1	94	4.3	49	2.0
60.0-69.9	260	5.4	42	7.1	218	5.1	96	7.3	59	1.7	198	6.6	62	1.6
70.0-79.9	275	14.6	66	22.7	209	12.0	126	17.5	38	5.3	235	17.0	40	0.0
> 80.0	109	36.7	33	39.4	76	35.5	66	43.9	5	20.0	104	37.5	5	20.0

Figure 2 Mortality rates by age at death or final visit observed in the total group and treatment categories. PDE5I: Phosphodiesterase 5-inhibitors.

However, our cohort is heterogeneous as it comprises men on varying treatments that influence mortality (Table 1). Figure 2 displays the relationship between mortality rates in the total group and in the different treatment and age categories (5 years selected due to lower numbers in the subgroups). As predicted in the logistic regression analyses (Table 2), statin, TRT and PDE5I treatments reduced the age related mortality, though the reduction differed in the age categories.

To further graphically demonstrate the impact of statin, TRT and PDE5I on mortality, the probability of mortality of each patient together with the 95%CI were estimated from the logistic regression analyses in Table 2. Figures 3 show the probability of mortality plotted against the age of the patient in the total cohort, by the treatment groups and by treatment combination (men on all 3 treatments *vs* not on any of the treatments). In the statin (Figure 3B) and TRT (Figure 3C) plots some overlap in the 95%CI is seen between treated compared to untreated men. For PDE5I (Figure 3D) and combination treatments (Figure 3E) no overlap of 95%CI values was observed after 50 years of age indicating the relationship between mortality and age is significantly altered.

DISCUSSION

In a recent longitudinal study we showed that in men

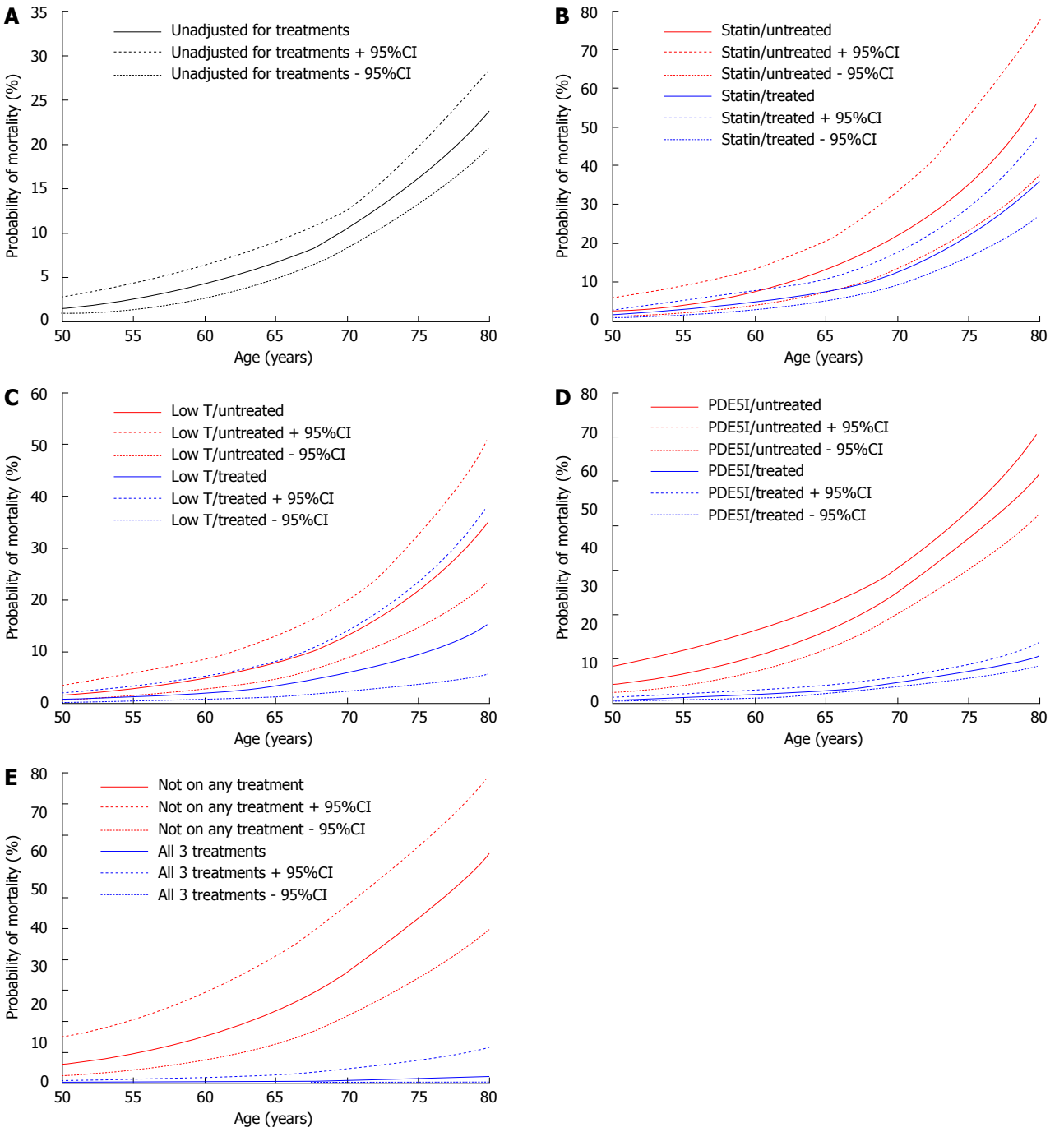


Figure 3 Association between probability of mortality and age. The estimated mortality probability and 95%CI from the fitted logistic regression (Table 2) were calculated from the logistic regression analyses seen in Table 2 and plotted against age at death or final visit in the following groups. Age was restricted to between 50-80 years due to reduced patient numbers in the treatment (Low T/treated and PDE5I/treated) groups (> 80 years) and the exponential pattern only being evident in the total group over the age of 50 years (Figure 1). A: Total group (from Model a in Table 2); B: Men stratified by statin treatment (from Model b in Table 2); C: Men stratified by testosterone treatment (from Model c in Table 2); D: Men stratified by PDE5I treatment (from Model d in Table 2); E: Men on all and none of the above treatments (from Model e in Table 2). PDE5I: Phosphodiesterase 5-inhibitors.

with T2DM, hypogonadism is associated with increased mortality compared to eugonadal men. Importantly TRT abolished this increase in mortality[19]. PDE5I (HR = 0.21, P = 0.009) and possibly statin (HR = 0.69, P = 0.086) use were also observed to reduce mortality[19]. Our aim in this paper was to determine how these three commonly used treatments influence the association between age and mortality in T2DM men. Our approach was to determine the probability of a patient in each treatment group living or dying at a particular age. Importantly, the Gompertz-Makeham law accurately describes the association between age and mortality in subjects aged approximately between 30-80 years, an age range that encompasses most of our study group.

Data on the United Kingdom Government Web Archive (http://webarchive.nationalarchives.gov.uk/20160

105160709/http://www.ons.gov.uk/ons/rel/vsob1/death-reg-sum-tables/2013/sty-mortality-rates-by-age.html - accessed 2016 Oct 24) shows firstly, the relevance of the Gompertz model; mortality rates between 1963 and 2013 demonstrate an exponential pattern similar to that described nearly 200 years ago by Gompertz. Secondly, the Archive data show that while the adult mortality rate had fallen during 1963-2013, possibly because of a reduction in CVD resulting from statin use and smoking cessation, it still demonstrated an exponential pattern in both genders.

In this study, we used a retrospective approach based on logistic regression to study the impact of treatment on the association between age and mortality. Accordingly, we compared results from this study with those from a previous prospective longitudinal analysis[19]. As expected, similar independent associations between the treatments and mortality were observed (Table 2, model e) enabling us to examine the impact of treatment on the relationship between age and probability of mortality. The relationship between age and mortality remained significant regardless of which (single or combination) treatment factors were added to regression models.

Life tables derived from data from an adult population will reflect a combination of phenotypes related to lifestyle, pathology, therapy and genetic factors that confer varying risks of dying at a particular age. Thus, we did not expect such a close fit (R^2 = 0.881) as that observed when the mortality rates of our total cohort were transformed logarithmically and plotted against age. It was tempting to carry out a similar exercise in the treatment and non-treatment subgroups but small patient numbers and low mortality rates in the testosterone and PDE5I treated men prevented this. Greater patient numbers with higher event rates would have permitted a study of these subgroups.

Using logistic regression to estimate probability of mortality for each individual patient allowed graphic demonstration of the impact of treatment on age, the most significant predictor of death. Whilst statin use and TRT did not show a statistically significant effect on the relationship between age and mortality, PDE5I treatment and combination (statin, TRT and PDE5I) treatment clearly did with no overlap of 95%CI. These results are compatible with studies showing that in T2DM patients, treatment with vardenafil results in improved endothelial parameters including flow-mediated dilation, interleukin-6 and testosterone levels[29] and indicate that a randomised controlled trial (RCT) is required for PDE5I and TRT in men with T2DM. It would be interesting if some of the large RCTs carried out showing statin-associated reductions in all-cause mortality such as 4S were analysed to establish ways in which the relationship between age and mortality may have been altered.

There are limitations to our study. The TRT arm was based on an intention to treat. The age of onset and duration of diabetes as well as exposure to statins and PDE5I were not documented. Data on the type of drug and dose was not completely recorded. We assumed that statin and PDE5I treatments were protocol driven. However, it is possible that patient selection, especially with PDE5I prescribing existed. It is believed however, that the principal reason for PDE5I prescribing is ED which has been established as a significant predictor of CVD and all-cause mortality.

Despite their limitations, our findings are important. We showed that mortality rates in men with T2DM follow the pattern described by Gompertz. We confirmed that statin, TRT and PDE5I reduce mortality in this cohort and have described how they influenced the relationship between age and mortality. We believe that our approach of communicating the effectiveness of an intervention by determining the probability of mortality at different ages is easy to understand and could be used by clinicians to improve patient compliance and lead to clinical benefit.

Our study examines the relationship between age and mortality in men with diabetes. Age was related to mortality in accordance with the Gompertz-Makeham law. We show that statin, TRT and PDE5I treatments impacted all-cause mortality and PDE5I treatment alone and in combination with statin and TRT significantly altered the relationship between age and mortality.

COMMENTS

Background

As far as the authors are aware the Gompertz-Makeham association has not been used to demonstrate the impact of medical treatments on the relationship between age and mortality.

Research frontiers

There is considerable debate regarding the effects of testosterone replacement therapy and phosphodiesterase 5-inhibitors (PDE5I) treatment in diabetic men. Although no randomised controlled studies exist longitudinal observational studies have shown potential benefits.

Innovations and breakthroughs

Having established that statin, PDE5I treatment and testosterone replacement therapy reduced mortality in the cohort, the studied the influence of these agents on this association. The data are presented in a novel graphical manner, clearly demonstrating the impact of these agents on mortality. The authors suggest the graphical illustrations in this paper will communicate the benefit of these interventions to patients and have a major positive bearing on patient compliance.

Peer-review

This paper is a very interesting retrospective study investigating whether the mortality rate follows the pattern described by Gompertz and estimating how testosterone status and treatments (statins, testosterone replacement therapy and phosphodiesterase 5 inhibitors) alter the mortality rate. A substantial and extremely meticulous work has been done and the findings are consistent.

REFERENCES

1 **Parsonnet J**, Greene KD, Gerber AR, Tauxe RV, Vallejo Aguilar OJ, Blake PA. Shigella dysenteriae type 1 infections in US travellers to Mexico, 1988. *Lancet* 1989; **2**: 543-545 [PMID: 2570242 DOI: 10.1098/rstb.2014.0379]

2 **Hallén A**. Makeham's addition to the Gompertz law re-evaluated. *Biogerontology* 2009; **10**: 517-522 [PMID: 18951143 DOI: 10.1007/s10522-008-9184-0]

3 **Vaupel JW**. Biodemography of human ageing. *Nature* 2010; **464**:

536-542 [PMID: 20336136 DOI: 10.1038/nature08984]
4 **Beltrán-Sáncheza H**, Crimmins E, Finch C. Early cohort mortality predicts the rate of aging in the cohort: a historical analysis. *J Dev Orig Health Dis* 2012; **3**: 380-386 [PMID: 23626899 DOI: 10.1017/S2040174412000281]
5 **Mulnier HE**, Seaman HE, Raleigh VS, Soedamah-Muthu SS, Colhoun HM, Lawrenson RA. Mortality in people with type 2 diabetes in the UK. *Diabet Med* 2006; **23**: 516-521 [PMID: 16681560 DOI: 10.1111/j.1464-5491.2006.01838.x]
6 **Hansen LJ**, Olivarius Nde F, Siersma V. 16-year excess all-cause mortality of newly diagnosed type 2 diabetic patients: a cohort study. *BMC Public Health* 2009; **9**: 400 [PMID: 19878574 DOI: 10.1186/1471-2458-9-400]
7 **Morgan CL**, Currie CJ, Peters JR. Relationship between diabetes and mortality: a population study using record linkage. *Diabetes Care* 2000; **23**: 1103-1107 [PMID: 10937505 DOI: 10.2337/diacare.23.8.1103]
8 **Nwaneri C**, Bowen-Jones D, Cooper H. Screening for type 2 diabetes and population mortality over 10 years. *Lancet* 2013; **381**: 901-902 [PMID: 23499033 DOI: 10.1016/S0140-6736(13)60665-0]
9 **Collins R**, Armitage J, Parish S, Sleigh P, Peto R. MRC/BHF Heart Protection Study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. *Lancet* 2003; **361**: 2005-2016 [PMID: 12814710 DOI: 10.1016/S0140-6736(03)13636-7]
10 **Colhoun HM**, Betteridge DJ, Durrington PN, Hitman GA, Neil HA, Livingstone SJ, Thomason MJ, Mackness MI, Charlton-Menys V, Fuller JH. Primary prevention of cardiovascular disease with atorvastatin in type 2 diabetes in the Collaborative Atorvastatin Diabetes Study (CARDS): multicentre randomised placebo-controlled trial. *Lancet* 2004; **364**: 685-696 [PMID: 15325833 DOI: 10.1016/S0140-6736(04)16895-5]
11 **Pyörälä K**, Pedersen TR, Kjekshus J, Faergeman O, Olsson AG, Thorgeirsson G. Cholesterol lowering with simvastatin improves prognosis of diabetic patients with coronary heart disease. A subgroup analysis of the Scandinavian Simvastatin Survival Study (4S) *Diabetes Care* 1997; **20**: 614-620 [PMID: 9096989 DOI: 10.2337/diacare.20.4.614]
12 **Hackett GI**, Cole NS, Deshpande AA, Popple MD, Kennedy D, Wilkinson P. Biochemical hypogonadism in men with type 2 diabetes in Primary Care Practice. *Br J Diabetes Vasc Dis* 2009; **5:** 226-231 [DOI: 10.1177/1474651409342635]
13 **Ma RC**, So WY, Yang X, Yu LW, Kong AP, Ko GT, Chow CC, Cockram CS, Chan JC, Tong PC. Erectile dysfunction predicts coronary heart disease in type 2 diabetes. *J Am Coll Cardiol* 2008; **51**: 2045-2050 [PMID: 18498959 DOI: 10.1016/j.jacc.2008.02.051]
14 **Dong JY**, Zhang YH, Qin LQ. Erectile dysfunction and risk of cardiovascular disease: meta-analysis of prospective cohort studies. *J Am Coll Cardiol* 2011; **58**: 1378-1385 [PMID: 21920268 DOI: 10.1016/j.jacc.2011.06.024]
15 **Gazzaruso C**, Solerte SB, Pujia A, Coppola A, Vezzoli M, Salvucci F, Valenti C, Giustina A, Garzaniti A. Erectile dysfunction as a predictor of cardiovascular events and death in diabetic patients with angiographically proven asymptomatic coronary artery disease: a potential protective role for statins and 5-phosphodiesterase inhibitors. *J Am Coll Cardiol* 2008; **51**: 2040-2044 [PMID: 18498958 DOI: 10.1016/j.jacc.2007.10.069]
16 **Fukui M**, Kitagawa Y, Nakamura N, Kadono M, Mogami S, Hirata C, Ichio N, Wada K, Hasegawa G, Yoshikawa T. Association between serum testosterone concentration and carotid atherosclerosis in men with type 2 diabetes. *Diabetes Care* 2003; **26**: 1869-1873 [PMID: 12766125 DOI: 10.2337/diacare.26.6.1869]
17 **Muraleedharan V**, Marsh H, Kapoor D, Channer KS, Jones TH. Testosterone deficiency is associated with increased risk of mortality and testosterone replacement improves survival in men with type 2 diabetes. *Eur J Endocrinol* 2013; **169**: 725-733 [PMID: 23999642 DOI: 10.1530/EJE-13-0321]
18 **Shores MM**, Smith NL, Forsberg CW, Anawalt BD, Matsumoto AM. Testosterone treatment and mortality in men with low testosterone levels. *J Clin Endocrinol Metab* 2012; **97**: 2050-2058 [PMID: 22496507 DOI: 10.1210/jc.2011-2591]
19 **Hackett G**, Heald AH, Sinclair A, Jones PW, Strange RC, Ramachandran S. Serum testosterone, testosterone replacement therapy and all-cause mortality in men with type 2 diabetes: retrospective consideration of the impact of PDE5 inhibitors and statins. *Int J Clin Pract* 2016; **70**: 244-253 [PMID: 26916621 DOI: 10.1111/ijcp.12779]
20 **Hackett G**, Cole N, Saghir A, Jones P, Strange RC, Ramachandran S. Testosterone undecanoate improves sexual function in men with type 2 diabetes and severe hypogonadism: results from a 30-week randomized placebo-controlled study. *BJU Int* 2016; **118**: 804-813 [PMID: 27124889 DOI: 10.1111/bju.13516]
21 **Francis SH**, Corbin JD. Molecular mechanisms and pharmacokinetics of phosphodiesterase-5 antagonists. *Curr Urol Rep* 2003; **4**: 457-465 [PMID: 14622499 DOI: 10.1007/s11934-003-0027-x]
22 **Vlachopoulos C**, Ioakeimidis N, Rokkas K, Stefanadis C. Cardiovascular effects of phosphodiesterase type 5 inhibitors. *J Sex Med* 2009; **6**: 658-674 [PMID: 19138362 DOI: 10.1111/j.1743-61 09.2008.01107.x]
23 **Anderson SG**, Hutchings DC, Woodward M, Rahimi K, Rutter MK, Kirby M, Hackett G, Trafford AW, Heald AH. Phosphodiesterase type-5 inhibitor use in type 2 diabetes is associated with a reduction in all-cause mortality. *Heart* 2016; **102**: 1750-1756 [PMID: 27465053 DOI: 10.1136/heartjnl-2015-309223]
24 **Hackett G**, Cole N, Bhartia M, Kennedy D, Raju J, Wilkinson P. Testosterone replacement therapy improves metabolic parameters in hypogonadal men with type 2 diabetes but not in men with coexisting depression: the BLAST study. *J Sex Med* 2014; **11**: 840-856 [PMID: 24308723 DOI: 10.1111/jsm.12404]
25 **Bhasin S**, Cunningham GR, Hayes FJ, Matsumoto AM, Snyder PJ, Swerdloff RS, Montori VM. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2010; **95**: 2536-2559 [PMID: 20525905 DOI: 10.1210/jc.2009-2354]
26 **Hackett G**, Cole N, Bhartia M, Kennedy D, Raju J, Wilkinson P, Saghir A. The response to testosterone undecanoate in men with type 2 diabetes is dependent on achieving threshold serum levels (the BLAST study). *Int J Clin Pract* 2014; **68**: 203-215 [PMID: 24355040 DOI: 10.1111/ijcp.12235]
27 **Hackett G**, Kell P, Ralph D, Dean J, Price D, Speakman M, Wylie K. British Society for Sexual Medicine guidelines on the management of erectile dysfunction. *J Sex Med* 2008; **5**: 1841-1865 [PMID: 18298470 DOI: 10.1111/j.1743-6109.2008.00773.x]
28 **Vermeulen A**, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 1999; **84**: 3666-3672 [PMID: 10523012 DOI: 10.1210/jcem.84.10.6079]
29 **Santi D**, Granata AR, Guidi A, Pignatti E, Trenti T, Roli L, Bozic R, Zaza S, Pacchioni C, Romano S, Nofer JR, Rochira V, Carani C, Simoni M. Six months of daily treatment with vardenafil improves parameters of endothelial inflammation and of hypogonadism in male patients with type 2 diabetes and erectile dysfunction: a randomized, double-blind, prospective trial. *Eur J Endocrinol* 2016; **174**: 513-522 [PMID: 26792933 DOI: 10.1530/EJE-15-1100]

P- Reviewer: Dimitriadis F, Georgescu A, Li HJ **S- Editor**: Qi Y
L- Editor: A **E- Editor**: Lu YJ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/

World J Diabetes 2017 March 15; 8(3): 112-119

DOI: 10.4239/wjd.v8.i3.112

ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Prospective Study

Role of angiotensin converting enzyme and angiotensinogen gene polymorphisms in angiotensin converting enzyme inhibitor-mediated antiproteinuric action in type 2 diabetic nephropathy patients

Neerja Aggarwal, Pawan Kumar Kare, Parul Varshney, Om Prakash Kalra, Sri Venkata Madhu, Basu Dev Banerjee, Anil Yadav, Alpana Raizada, Ashok Kumar Tripathi

Neerja Aggarwal, Parul Varshney, Om Prakash Kalra, Sri Venkata Madhu, Anil Yadav, Alpana Raizada, Department of Medicine, University College of Medical Sciences (University of Delhi) and Guru Teg Bahadur Hospital, Delhi 110095, India

Pawan Kumar Kare, Basu Dev Banerjee, Ashok Kumar Tripathi, Biochemistry and Immunology Laboratory, Department of Biochemistry, University College of Medical Sciences (University of Delhi) and Guru Teg Bahadur Hospital, Delhi 110095, India

Om Prakash Kalra, Pt. B. D. Sharma University of Health Sciences, Rohtak 124001, India

Author contributions: Kalra OP, Madhu SV, Banerjee BD and Tripathi AK were involved in planning and designing the research work; Kalra OP, Madhu SV, Yadav A and Raizada A contributed to the enrollment and medication of patients; Aggarwal N, Kare PK and Varshney P were involved in the biochemical investigations and data collection; Aggarwal N carried out data interpretation and drafted the manuscript; Tripathi AK, the corresponding author, was involved in overall supervision and revision of the manuscript.

Supported by Department of Biotechnology, Government of India, New Delhi (DBT Project), No. BT/PR 4640/MED/30/716/2012.

Institutional review board statement: All procedures performed in the study involving human participants were reviewed and approved by UCMS and GTB hospital ethic committee.

Informed consent statement: Informed consent was obtained prior to enrollment from all individual participants included in the study.

Conflict-of-interest statement: The authors of this manuscript have no conflicts of interest to disclose.

Data sharing statement: There is no additional data available.

Manuscript source: Invited manuscript

Correspondence to: Ashok Kumar Tripathi, PhD, Professor, Biochemistry and Immunology Laboratory, Department of Biochemistry, University College of Medical Sciences (University of Delhi) and Guru Teg Bahadur Hospital, Dilshad Garden, Delhi 110095, India. aktripathiucms@gmail.com
Telephone: +91-11-22582972

Received: July 11, 2016
Peer-review started: July 14, 2016
First decision: September 12, 2016
Revised: October 6, 2016
Accepted: November 21, 2016
Article in press: November 23, 2016
Published online: March 15, 2017

Abstract

AIM

To investigate the role of genetic variants of angiotensin converting enzyme (ACE) and angiotensinogen (AGT) genes in the antiproteinuric efficacy of ACE inhibitor therapy in diabetic nephropathy (DN) patients.

METHODS

In the present study, 270 type 2 diabetes mellitus patients with nephropathy were enrolled and treated with ACE inhibitor (ramipril) and followed at 6 mo for renal function and albumin excretion by estimating serum creatinine, end stage renal disease, and albumin/creatinine ratio (ACR) in urine. Genotyping of ACE I/D and AGT M235T polymorphisms were performed by using primer specific polymerase chain reaction (PCR) and PCR-RFLP techniques, respectively.

RESULTS

Forty-eight percent of DN patients (responders) benefited with respect to proteinuria from ACE inhibitor therapy at 6 mo follow-up. A significant reduction in ACR was observed after 6 mo treatment with ACE inhibitor irrespective of whether DN patients were micro-albuminuric (≥ 30 and < 300 mg/g creatinine) or macro-albuminuric (≥ 300 mg/g creatinine) at the time of enrollment. However, macro-albuminuric patients (55%) showed better response to therapy. A reduction in urinary ACR was found independent of genotypes of ACE I/D and AGT M235T polymorphisms although macro-albuminuric patients having TT genotype showed statistically insignificant increased response (72%).

CONCLUSION

ACE inhibitor therapy reduced urinary ACR by ≥ 30% in 50% of DN patients and the response is independent of ACE I/D and AGT M235T polymorphisms.

Key words: Diabetic nephropathy; Angiotensin converting enzyme inhibitor therapy; Renin-angiotensin-aldosterone system gene polymorphisms; Responder; Urinary albumin/creatinine ratio; Albuminuria

Core tip: Angiotensin converting enzyme (ACE) inhibitors are used as standard therapy in patients with diabetic nephropathy (DN) and reported to have reno-protective effect in these patients; however, the response to ACE inhibitor therapy is not uniform in all patients. We investigated whether ACE I/D and angiotensinogen gene (AGT) M235T polymorphisms of genes of the renin-angiotensin-aldosterone system are associated with variable response to ACE inhibitors in DN patients. ACE inhibitor treatment in DN patients caused a significant reduction in urinary protein excretion and was found independent of ACE I/D and AGT M235T polymorphisms.

Aggarwal N, Kare PK, Varshney P, Kalra OP, Madhu SV, Banerjee BD, Yadav A, Raizada A, Tripathi AK. Role of angiotensin converting enzyme and angiotensinogen gene polymorphisms in angiotensin converting enzyme inhibitor-mediated antiproteinuric action in type 2 diabetic nephropathy patients. *World J Diabetes* 2017; 8(3): 112-119 Available from: URL: http://www.wjgnet.com/1948-9358/full/v8/i3/112.htm DOI: http://dx.doi.org/10.4239/wjd.v8.i3.112

INTRODUCTION

Diabetic nephropathy (DN) is a clinical syndrome that occurs approximately in 20%-30% of patients with diabetes mellitus (DM). Nephropathy gradually progresses and makes the patient dependent on renal replacement therapy. DN patients clinically present with persistent micro-albuminuria (≥ 30 to 299 mg/g creatinine) which subsequently progresses to macro-albuminuria (≥ 300 mg/g creatinine)[1]. Later severity of the disease is characterized by a fall in estimated glomerular filtration rate (eGFR) as a consequence of renal impairment, ultimately leading to end stage renal disease (ESRD)[2]. Various factors including poor glycemic control, family history of diabetes or hypertension may predispose to the development of DN; however; not all DM patients tend to develop nephropathy[3].

The renin-angiotensin-aldosterone system (RAAS), which plays an important role in regulating blood pressure, is involved in the pathophysiology of renal complications including DN. Polymorphisms of various genes of RAAS, particularly angiotensin converting enzyme (ACE) and angiotensinogen (AGT) genes, have been strongly implicated in the development and progression of nephropathy[4,5]. ACE is a zinc-dependent di-peptidase enzyme which catalyzes the conversion of inactive angiotensin (angiotensin-Ⅰ) to angiotensin-Ⅱ[6]. The *ACE* gene is located at the 17q23 locus and known to be associated with the pathogenesis of DN, including progression to overt proteinuria. The *ACE* gene is highly polymorphic in nature. Of the 160 polymorphisms known, insertion/deletion (*I/D*) polymorphism is the most studied as it affects ACE enzyme activity in blood. *I/D* polymorphism involves the presence or absence of a 287 bp Alu repeat in intron 16 of the gene. It has been observed that DD genotype is associated with higher ACE activity and II genotype is associated with the lowest ACE activity[7].

The *AGT* gene (*rs 699*) is located at chromosome 1 and consists of five exons, and it has more than 23 variants[8]. The common polymorphism of the *AGT* gene is *M235T*, which encodes threonine instead of methionine at position 235 in exon 2[9]. T allele of the *M235T* variant is associated with a higher plasma AGT level[10].

A number of drugs that block the RAAS like ACE inhibitors and angiotensin receptor blockers (ARB) are often prescribed to control hypertension; in addition, these drugs are known to control proteinuria either alone or in combination in DN patients[11]. However, the reno-protective response to ACE inhibitor therapy is not uniform in all patients. The reasons behind the uneven antiproteinuric response to these drugs are not completely understood. The polymorphisms of genes of RAAS may be possibly involved in this process.

Despite several studies on association of *ACE* and *AGT* gene polymorphisms with ACE inhibitor treatment in type 2 DM (T2DM) patients with nephropathy, no substantial data are available on the role of *ACE* and *AGT* gene polymorphisms in antiproteinuric efficacy of ACE

inhibitors in the Indian context. In the present study, we examined the association of *ACE* and *AGT* gene polymorphisms with antiproteinuric response to ACE inhibitor therapy in north Indian type 2 diabetic patients with nephropathy.

MATERIALS AND METHODS

Subjects

This study was designed as a single arm prospective longitudinal study to evaluate the antiproteinuric effect of ACE inhibitor therapy based on change in albumin/creatinine ratio (ACR), with the baseline data serving as reference values (control). The required number of cases for 80% power at 5% type Ⅰ error in detecting a reduction of proteinuria to at least 30% of pretreatment value for a given odds ratio of 1.5 is 221, based on the frequency of mutant *ACE* gene allele in the Asian population as 40%[12]. In order to accommodate drop out during the course of the study, we recruited 270 patients with T2DM having persistent microalbuminuria (30-300 mg/g creatinine) or overt albuminuria (> 300 mg/g creatinine), of whom 18 could not complete the follow-up. The patients were enrolled from Department of Medicine, Diabetic and Nephrology Clinic at Guru Teg Bahadur Hospital, Delhi, India. Patients having an age between 30 to 65 years and a duration of diabetes ⩾ 5 years, with the evidence of diabetic retinopathy and stages 1 to 3 chronic kidney disease (CKD), were recruited. Patients intolerant to ACE inhibitors, pregnant or lactating women, patients taking aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs) were excluded from the study. Diagnosis of DM was based upon the 2012 American Diabetes Association (ADA) guidelines. Patients having evidence of 1+ or more proteinuria by urinary dipstick test were included in the study. In addition, patients with dipstick negative proteinuria were screened by urinary dipstick for the presence of microalbumin. Patients with evidence of micro-albuminuria or overt proteinuria on two separate occasions at least 6 wk apart were included in the study and assessed for urinary ACR.

The study was approved by Institutional Ethics Committee-Human Research (IEC-HR) of University College of Medical Sciences and written informed consent was obtained from all patients. All enrolled patients were under satisfactory glycemic control and were under well-controlled blood pressure. The patients were followed after 6 mo of initiation of ACE inhibitor therapy. All were treated initially with ramipril 5 mg/d along with anti-diabetic therapy. The dose was up-titrated to a maximum of 20 mg/d at one or two equally divided doses.

Clinical response assessment

The decrease in urinary ACR (ACR%) was calculated as (baseline value - follow-up value) × 100/baseline value.

Patients were classified as responders when they had a decrease in urinary ACR ⩾ 30% or as non-responders when they had a decrease in urinary ACR > 30% at the end of 6 mo follow-up[13,14].

Measurement of biochemical parameters

Blood samples (5 mL) were collected for biochemical analysis and genotype study. Blood was centrifuged at 1000 g for 15 min for serum separation. Serum samples were frozen at -80 ℃ until assayed. All parameters were determined within a month after sample collection. Morning spot urine samples were collected for urine albumin and urine creatinine tests.

The plasma glucose level was measured by glucose oxidase-peroxidase method and quantified spectrophotometrically at 500 nm. HbA1c was estimated by micro-column based technique and quantified spectrophotometrically at 500 nm. Total cholesterol (TC), serum sodium, potassium and hemoglobin were determined using routine clinical assays in hospital laboratory. Average of three blood pressure readings taken 15 min apart was calculated, and all patients underwent fundus examination for the detection of diabetic retinopathy.

Urine and serum creatinine levels were estimated by alkaline picrate Jaffe's method (kinetic method). Urine albumin was measured by an immuno-turbidometric assay (Nephelometer, Nephstar) after calibration of the instrument by the standard provided. The minimum sensitivity is 10 mg/L. The result is expressed as ACR in terms of mg/g creatinine.

Determination of genotypes

ACE I/D gene polymorphism: The *ACE* gene (*rs 4646994*) *I/D* polymorphism was determined by polymerase chain reaction (PCR) using a flanking primer pair that recognizes the insertion-specific sequence. The 25 μL PCR reaction mixture contained 100 ng of genomic DNA and amplification buffer containing 20 mmol/L Tris (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 200 μmol/L of dNTPs, 10 pmol of each primer, and 1.0 U of Taq DNA polymerase (RBC, India). The DNA was amplified by cycling at 94 ℃ for 2 min, at 60 ℃ for 45 s, and at 72 ℃ for 2 min (Eppendorf PCR machine, Germany). After 30 cycles, the reaction was extended for an additional 8 min at 72 ℃. The oligonucleotide sequences of the primers were: 5'-CTGGAGACCACTCCCATCCTTTCT-3' and 5'-GATGTGGCCATCACATTCGTCAGAT-3'. The PCR products were separated by 1.5% agarose gel electrophoresis, and a 490 bp band with insertion (I allele) and a 190 bp band with deletion (D allele) were visualized with ethidium bromide staining in the UVP Bio-Documentation System.

AGT M235T gene polymorphism: The *AGT* gene (*rs 699*) *M235T* polymorphism was determined by PCR-restriction fragment length polymorphism (PCR-RFLP) assay. The 25 μL PCR reaction mixture contained 100 ng of genomic DNA and amplification buffer containing 20 mmol/L Tris (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 200 μmol/L of dNTPs, 10 pmol of each primer, and 1.0 U of Taq DNA polymerase (RBC, India). The DNA was amplified by cycling at 94 ℃ for 1 min, at 68 ℃ for 45 s, and at 72 ℃

Table 1 Demographic and clinical characteristics of patients

Parameter	Type 2 diabetes mellitus with nephropathy
Number of patients (*n*)	270
Gender (male/female)	128/142
Age (yr)	52.23 ± 6.01[1]
Duration of diabetes (yr)	8.31 ± 3.09[1]
Family history of diabetes (yes/no)	105/165
Family history of hypertension (yes/no)	63/207
Medications	
Insulin (yes/no)	115/155
Metformin (yes/no)	153/117
Glimiperide (yes/no)	129/141

[1]Data are presented as mean ± SD.

for 2 min (Eppendorf PCR machine, Germany). After 30 cycles, the reaction was extended for an additional 10 min at 72 ℃. The oligonucleotide sequences of the primers were: 5′-CCGTTTGTGCAGGGCCTGGCTCTCT-3′ and 5′-CAGGGTGCTGTCCACACTGGACCCC-3′.

The PCR product was digested with restriction enzyme Tth111 I (Fermentas) to identify the M/T polymorphism at 37 ℃ for 16 h. Digested DNA fragment products were separated by 2% agarose gel electrophoresis and visualized by ethidium bromide staining. The presence of an uncut 165 bp band indicated homozygous MM genotype, 141 bp and 24 bp bands indicated TT homozygous genotype, and 165 bp, 141 bp and 24 bands indicated MT heterozygous genotype.

Statistical analysis

The statistical methods of this study were reviewed by Department of Biostatistics, UCMS and GTB Hospital, Delhi, India. Data of all the parameters were collected at enrollment and at 6 mo after ramipril treatment. Analyses of obtained data were performed by using SPSS, version 20.0. *P*-values < 0.05 were considered significant. χ^2 test was applied to compare genotype data of *ACE* and *AGT* genes in all groups. For biochemical parameters, paired student's *t*-test was applied to compare the baseline values with the values obtained at 6 mo. ACR values follows the skewed distribution, hence we applied non-parametric method (Wilcoxon-signed rank test) to compare the baseline ACR values with the values obtained at 6 mo.

RESULTS

Demographic and biochemical data at baseline and at 6 mo after ACE inhibitor therapy

The demographic and biochemical data are listed in Tables 1 and 2. The age of the patients ranged from 30 to 60 years. The duration of diabetes ranged from 5 years to 20 years and mean duration of diabetes was 8.31 years. Approximately 39% of enrolled patients had a family history of diabetes and 23% had a family history of hypertension. Biochemical data before treatment and after 6 mo of treatment with ramipril are listed in Table 2. There was no significant change in blood urea, serum sodium, serum potassium, fasting plasma glucose, post prandial plasma glucose, systolic and diastolic blood pressure, hemoglobin or HbA1c level after follow-up. Also, the differences in serum creatinine and eGFR levels after treatment were not statistically significant.

Antiproteinuric effect of ACE inhibitor therapy

The antiproteinuric effect of ACE inhibitor therapy was evaluated by urinary ACR values. Patients with a decrease of more than 30% in ACR values were considered as responders to ACE inhibitor treatment. ACR values of enrolled patients at baseline varied widely and ranged from 30 to 14573 mg/g creatinine. An overall significant decrease in ACR values was observed after ACE inhibitor treatment as compared to baseline values (Table 3). Taken together, 48% of enrolled patients were found as responders to ACE inhibitor therapy. Subsequently, based on the ACR, patients were grouped as micro-albuminuric (ACR $\geqslant$ 30 and $\leqslant$ 300 mg/g creatinine) and macro-albuminuric (ACR > 300 mg/g creatinine). A significant decrease in ACR was observed in both the micro- and macro-albuminuric DN groups. In the micro-albuminuric DN group (n = 170), the percentage of responders was 45% whereas in the macro-albuminuric group (n = 82), the percentage was 55% at 6 mo follow-up.

Distribution of genotypes of ACE and AGT genes

ACE *I/D* polymorphism was studied by sequence specific PCR method and AGT *M235T* polymorphism was studied by PCR-RFLP method. Genotype distribution and allele frequency for *ACE* and *AGT* genes are listed in Table 4. Distribution of all genotypes was in Hardy-Weinberg equilibrium for all the subgroups of *ACE* and *AGT* genes. For *ACE* gene, the genotype frequency of II, ID, and DD was found to be 31%, 53% and 16%, respectively. For *AGT* gene, the genotype frequency of MM, MT, and TT was found to be 25%, 53% and 22%, respectively.

ACE and AGT polymorphisms and response to ACE inhibitor therapy

Table 5 shows the genotype distribution of DN patients based on the response to ACE inhibitor therapy. No significant change in the genotype distribution was observed among responders and non-responders with regard to *ACE* and *AGT* genes. When the patients were grouped as micro- and macro-albuminuric based on their ACR values (Table 6), no inter-genotype differences were observed in subgroups. However, macro-albuminuric patients carrying ACE *I/D* genotypes were responding in a better way to therapy compared with micro-albuminuric patients. Seventy-two percent of macro-albuminuric patients having TT genotype responded to therapy, although the difference was not statistically significant.

DISCUSSION

In the present study, we examined the antiproteinuric

Table 2 Biochemical parameters before and after treatment with angiotensin converting enzyme inhibitor

Parameter	Baseline[1]	6 mo[1,2]	*P*-value
No. of patients	*n* = 252	*n* = 252	
Blood urea (mmol/L)	2.22 ± 0.86	2.01 ± 0.77	0.661
Serum creatinine (μmol/L)	95.47-30	99-28.28	0.068
Serum sodium (mmol/L)	139.47 ± 4.11	135.14 ± 3.88	0.512
Serum potassium (mmol/L)	4.32 ± 0.65	4.30 ± 0.52	0.141
eGFR (MDRD) mL/min per 1.73 m^2	73.65 ± 24.71	68.90 ± 24.44	0.081
eGFR (EPI) mL/min per 1.73 m^2	73.40 ± 22.8	70.56 ± 21.30	0.07
Fasting plasma glucose (mmol/L)	7.63 ± 0.60	6.693 ± 0.81	0.08
Post-prandial plasma glucose (mmol/L)	10.33 ± 1.62	8.52 ± 1.3	0.076
HbA1c (%)	6.52 ± 1.71	6.1 ± 1.14	0.06
Hemoglobin (g/L)	123.8 ± 23	111.2 ± 31	0.65
Systolic blood pressure (mmHg)	132.30 ± 13.67	130.12 ± 10.46	0.71
Diastolic blood pressure (mmHg)	86.10 ± 10.03	84.07 ± 8.32	0.68

[1]Data are presented as mean ± SD; [2]*P* > 0.05. HbA1c: Hemoglobin A1c; eGFR: Estimated glomerular filtration rate.

Table 3 Responders and non-responders before and after treatment with angiotensin converting enzyme inhibitor therapy

Patients	Urinary ACR at baseline[1]	Urinary ACR at 6 mo	*P*-value	R^2 (%)	NR (%)
Overall (*n* = 252)	185.97 (55.66-222.20)	118.64 (96.24-146.26)	< 0.001	121	131
Micro-albumin (*n* = 170)	78.79 (71.30-87.07)	53.67 (44.46-64.79)	< 0.001	76	94
Macro-albumin (*n* = 82)	1068.7 (879.62–1298.28)	596.45 (451.60-787.68)	< 0.001	45	37

[1]Median (IQR); [2]A decline of > 30% in ACR value at 6 mo is considered as R. R: Responders; NR: Non-responders; ACR: Albumin/creatinine ratio.

Table 4 Genotype distributions and allele frequency for angiotensin converting enzyme and angiotensinogen gene polymorphisms

Gene	*n* = 252	Genotype/allele	Percentage (%)
ACE (I/D)	Genotypic	II	31
	frequency	ID	53
		DD	16
	Allele	I	57
	frequency	D	43
AGT (M235T)	Genotypic	MM	25
	frequency	MT	53
		TT	22
	Allele	M	51
	frequency	T	49

ACE: Angiotensin converting enzyme; AGT: Angiotensinogen.

effect of ACE inhibitor (ramipril) in DN patients by following urinary ACR. ACE inhibitors are commonly used for inhibition of the RAAS and are known to have renoprotective efficacy in both diabetic and non-diabetic kidney diseases[15] and antiproteinuric efficacy of ACE inhibitors are more pronounced than any other antihypertensive drugs[16]. However, there are variable responses regarding antiproteinuric efficacy of RAAS blockers among patients and a 20%-80% reduction was observed[17]. In the present study, overall we observed a 36% reduction in ACR values and about 48% of patients responded to therapy. Our finding is in accordance with previous studies showing overall decrease in albumin excretion after treatment with ACE inhibitor[13,18-20]. According to the NKF KDOQI guidelines[14], ACE inhibitors reduced protein excretion by approximately 35% to 40%, which is greater than other antihypertensive agents when the effect of blood pressure has been taken into account. Hence, in the present study patients with an ACR change ⩾ 30% were considered as responders to ACE inhibitor therapy. When subdividing our study subjects as micro- and macro-albuminuric, it was observed that 55% of patients with macro-albuminuria responded in a better way to ACE inhibitor therapy. Earlier antiproteinuric effect of ACE inhibitor has been shown to be more pronounced in macro-albuminuric patients[21,22]. The mechanism leading to the antiproteinuric effect of ACE inhibitors has not been elucidated fully. However, it is thought that ACE inhibitors cause efferent arteriolar vasodilation of glomerulus and thereby decrease the intraglomerular hypertension, leading to anti-proteinuric effect[23]. Recently it has been shown that ACE inhibitors ameliorate the glomerular membrane size-selective dysfunction, thus resulting in anti-proteinuric effect[24].

In order to find out the reason behind differential responses to ACE inhibitor therapy in DN patients, we studied the polymorphisms of *ACE* and *AGT* genes as these polymorphisms are strongly associated with the progression of DN. The genotype distribution of *ACE* gene observed in our study subjects is in line with most of the previous studies on the Indian population[25,26].

In the present study, the percentage of responders did not differ significantly with regard to *ACE I/D* genotypes, indicating that the antiproteinuric effect of ACE inhibitors is independent of *ACE* genotype. Similarly, the finding that the anti-proteinuric effect of ACE inhibitors is independent of *ACE* genotypes has been reported by

Table 5 Genotypic distribution of responders and non-responders

Gene	Genotype	No. of patients (n = 252)	At 6 mo follow-up		P-value[1]
			R (%) (n = 121)	NR (%) (n = 131)	
ACE (I/D)	II	78	38 (49)	40 (51)	0.893
	ID	133	62 (47)	71 (53)	
	DD	41	21 (51)	20 (49)	
AGT (M235T)	MM	61	34 (56)	27 (44)	0.369
	MT	134	59 (44)	75 (56)	
	TT	57	28 (49)	29 (51)	

[1]P > 0.05: Comparison between R or NR. ACE: Angiotensin converting enzyme; AGT: Angiotensinogen; R: Responders; NR: Non-responders.

Table 6 Genotypic distribution of responders and non-responders having micro-/ macro-albuminuria

Gene	Genotype (n = 252)	Micro-albuminuric group (n = 170)			Macro-albuminuric group (n = 82)		
		R (%) (n = 76)	NR (%) (n = 94)	P-value[1]	Rc (%) (n = 45)	NR (%) (n = 37)	P-value[2]
ACE (I/D)	II	23 (45)	28 (55)	0.974	15 (56)	12 (44)	0.636
	ID	42 (44)	53 (56)		20 (53)	18 (47)	
	DD	11 (49)	13 (54)		10 (59)	7 (41)	
AGT (M235T)	MM	25 (60)	17 (40)	0.11	9 (47)	10 (53)	0.201
	MT	36 (40)	53 (60)		23 (51)	22 (49)	
	TT	15 (38)	24 (62)		13 (72)	5 (28)	

[1]P > 0.05: Comparison between responders and non-responders to therapy in micro-albuminuric group; [2]P > 0.05: Comparison between responders and non-responders to therapy in macro-albuminuric group. ACE: Angiotensin converting enzyme; AGT: Angiotensinogen; R: Responders; NR: Non-responders.

several authors[14,27,28]. So *et al*[29] have reported that *ACE* Ⅱ genotype with a cumulative genetic risk score of < 1 in normoalbuminuricT2DM patients, is coupled with better response to ACE inhibitors, although no significant difference was found in renoprotective effect of ACE inhibitor therapy based on *ACE I/D* genotypes after 3 years of follow-up. The antiproteinuric effect of RAAS inhibitors in patients with macro-albuminuria is also found to be independent of *ACE I/D* genotypes[30]. However, there are a number of controversies about the association of *ACE I/D* genotypes with the therapeutic efficacy of ACE inhibitors. In Korean and Caucasian patients, DD genotype has been shown to be more responsive to ACE inhibitor therapy[31,32]. However, Japanese, European and Caucasian DN patients carrying II allele exhibit better reno-protection to ACE inhibitor therapy[33-35].

Another important gene of the RAAS is *AGT*, and *M235T* polymorphism influences the risk of nephropathy in T2DM patients[36,37]. Frequencies of M/T genotypes of the *AGT* gene in our study are similar to those reported by several other studies in different populations[36,38-41]. We observed that the percentage of responders did not differ significantly in different genotypes of the *AGT* gene, as compared to non-responders. This indicates that the antiproteinuric effect of ACE inhibitors is independent of genotypes of the *AGT* gene. When patients were subdivided as micro- and macro-albuminuric, we observed that macro-albuminuric patients carrying TT genotype showed better antiproteinuric response to ACE inhibitor therapy, although the result was not statistically significant. No significant reports are available on *AGT M235T* gene polymorphism and antiproteinuric response to ACE inhibitor therapy. Similar to our finding, reports by several authors failed to show any significant association between *AGT* polymorphism and diabetic chronic kidney disease[40,41]. Also no association was reported between *AGT M235T* genotypes and reduction in albumin excretion after ACE inhibitor treatment[29]. However, Narita *et al*[41] concludes that the therapeutic efficacy of ACE inhibitors or ARBs is influenced by *AGT M235T* genotypes in patients with IgA nephropathy.

Our study has several limitations. Patients were given different doses of ramipril as per their requirement of dose titration. In addition, short duration of follow-up period as well as heterogeneity in gender may also have hindered the significant association of *ACE I/D* or *AGT M235T* genotypes.

In conclusion, ACE inhibitor treatment in DN patients appears to cause a significant reduction in urinary protein excretion and macro-albuminuric patients exhibit better response. The antiproteinuric effect of ACE inhibitor therapy in patients is independent of *ACE I/D* and *AGT M235T* genotypes. Long term follow-up of larger populations with ACE inhibitor therapy may validate the present findings.

COMMENTS

Background

Angiotensin converting enzyme (ACE) inhibitors are the standard therapy for patients with hypertension, proteinuria and kidney diseases. The use of ACE inhibitors delays the progression of diabetic and non-diabetic kidney diseases. Various polymorphisms of the renin-angiotensin-aldosterone system (RAAS) have been implicated in the pathology of diabetic nephropathy. Of these,

polymorphism of the *ACE* gene is the most important. The current study was designed to evaluate the therapeutic efficacy of ACE inhibitor in terms of proteinuria and the role of *ACE* and *AGT* gene polymorphisms in ACE inhibitor-mediated antiproteinuric response in diabetic nephropathy patients.

Research frontiers

Patients on ACE inhibitor therapy have improved proteinuria. In this study, the authors observed that *ACE* and *AGT* gene polymorphisms do not have any role in reducing albuminuria in patients with diabetic nephropathy.

Innovations and breakthroughs

The literature suggests a mixed role of *ACE* gene polymorphisms in reno-protective action in diabetic patients. However, the present study suggests no role of *ACE I/D* and *AGT M235T* gene polymorphisms in modulating the renoprotective efficacy of ACE inhibitors in terms of reducing albuminuria in diabetic nephropathy patients.

Applications

The authors' study provides additional evidence supporting the therapeutic role of ACE inhibitors in reducing albuminuria. They conclude that genotypes of various genes of RAAS are not responsible for non-uniform response to ACE inhibitors in DN patients.

Terminology

Diabetic nephropathy: It is the damage to kidneys due to diabetes; Polymorphism: The presence of genetic variation within a population.

Peer-review

This is a good paper.

REFERENCES

1 **Molitch ME**, DeFronzo RA, Franz MJ, Keane WF, Mogensen CE, Parving HH, Steffes MW. Nephropathy in diabetes. *Diabetes Care* 2004; **27** Suppl 1: S79-S83 [PMID: 14693934 DOI: 10.2337/diacare.27.2007.S79]

2 **Dabla PK**. Renal function in diabetic nephropathy. *World J Diabetes* 2010; **15**: 48-56 [PMID: 21537427 DOI: 10.4239/wjd.v1.i2.48]

3 **Kalra OP**, Datta SK, Kumar S. Genetic basis of diabetic nephropathy. *Med Up* 2010; **20**: 695-701

4 **Hadjadj S**, Tarnow L, Forsblom C, Kazeem G, Marre M, Groop PH, Parving HH, Cambien F, Tregouet DA, Gut IG, Théva A, Gauguier D, Farrall M, Cox R, Matsuda F, Lathrop M, Hager-Vionnet N. Association between angiotensin-converting enzyme gene polymorphisms and diabetic nephropathy: case-control, haplotype, and family-based study in three European populations. *J Am Soc Nephrol* 2007; **18**: 1284-1291 [PMID: 17376814 DOI: 10.1681/ASN.2006101102]

5 **Ilić V**, Ilić M, Soldatović I, Popović S, Magić Z. Association of renin-angiotensin system genes polymorphism with progression of diabetic nephropathy in patients with type 1 diabetes mellitus. *Vojnosanit Pregl* 2014; **71**: 627-633 [PMID: 25109108 DOI: 10.2298/VSP1407627I]

6 **Gesualdo L**, Grandaliano G, Mascia L, Pertosa G, Schena FP. Acute renal failure in critically ill patients. *Intensive Care Med* 1999; **25**: 1188-1190 [PMID: 10551984 DOI: 10.1007/s001340051037]

7 **Rahimi Z**. ACE insertion/deletion (I/D) polymorphism and diabetic nephropathy. *J Nephropathol* 2012; **1**: 143-151 [PMID: 24475405 DOI: 10.5812/nephropathol.8109]

8 **Say YH**, Ling KH, Duraisamy G, Isaac S, Rosli R. Angiotensinogen M235T gene variants and its association with essential hypertension and plasma renin activity in Malaysian subjects: a case control study. *BMC Cardiovasc Disord* 2005; **5**: 7 [PMID: 15811183 DOI: 10.1186/1471-2261-5-7]

9 **Gaillard I**, Clauser E, Corvol P. Structure of human angiotensinogen gene. *DNA* 1989; **8**: 87-99 [PMID: 2924688 DOI: 10.1089/dna.1.1989.8.87]

10 **Jeunemaitre X**, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, Hunt SC, Hopkins PN, Williams RR, Lalouel JM. Molecular basis of human hypertension: role of angiotensinogen. *Cell* 1992; **71**: 169-180 [PMID: 1394429 DOI: 10.1016/0092-8674(92)90275-H]

11 **Tobli JE**, Bevione P, Di Gennaro F, Madalena L, Cao G, Angerosa M. Understanding the mechanisms of proteinuria: therapeutic implications. *Int J Nephrol* 2012; **2012**: 546039 [PMID: 22844592 DOI: 10.1155/2012/546039]

12 Hypertension and the ACE Gene-GB Healthwatch. Available from: URL: http//www.gbhealthwatch.com/GND-Hypertension-ACE.php

13 **Bakris GL**. Slowing nephropathy progression: focus on proteinuria reduction. *Clin J Am Soc Nephrol* 2008; **3** Suppl 1: S3-10 [PMID: 18178794 DOI: 10.2215/CJN.03250807]

14 **National Kidney Foundation**. K/DOQI Clinical Practice Guidelines on Hypertension and Antihypertensive Agents in Chronic Kidney Disease, 2004. Available from: URL: http//www2.kidney.org/professionals/kdoqi/guidelines_bp/guide_11.htm

15 **Narita I**, Goto S, Saito N, Song J, Omori K, Kondo D, Sakatsume M, Gejyo F. Renoprotective efficacy of renin-angiotensin inhibitors in IgA nephropathy is influenced by ACE A2350G polymorphism. *J Med Genet* 2003; **40**: e130 [PMID: 14684698 DOI: 10.1136/jmg.40.12.e130]

16 **Han SY**, Kwon YJ, Jo SK, Shin JH, Cha DR, Cho WY, Pyo HJ, Kim HK. ACE gene polymorphism and renal responsiveness to ACE inhibitors in IgA nephropathy patients. *Korean J Intern Med* 2000; **15**: 13-18 [PMID: 10714086 DOI: 10.3904/kjim.2000.15.1.13]

17 **Ha SK**. ACE insertion/deletion polymorphism and diabetic nephropathy: clinical implications of genetic information. *J Diabetes Res* 2014; **2014**: 846068 [PMID: 25587546 DOI: 10.1155/2014/846068]

18 **Li P**, Chen Y, Liu J, Hong J, Deng Y, Yang F, Jin X, Gao J, Li J, Fang H, Liu G, Shi L, Du J, Li Y, Yan M, Wen Y, Yang W. Efficacy and safety of tangshen formula on patients with type 2 diabetic kidney disease: a multicenter double-blinded randomized placebo-controlled trial. *PLoS One* 2015; **10**: e0126027 [PMID: 25938778 DOI: 10.1371/journal.pone.0126027]

19 **Remuzzi G**, Macia M, Ruggenenti P. Prevention and treatment of diabetic renal disease in type 2 diabetes: the BENEDICT study. *J Am Soc Nephrol* 2006; **17**: S90-S97 [PMID: 16565256 DOI: 10.1681/ASN.2005121324]

20 **Van de wal RMA**, Gansevoort RT, Harst PV, Boomsma F, Plokker HWT, Veldhuisen DJV, De Jong PE, van Gilst WH, Voors AA. Predictors of Angiotensin-Converting Enzyme Inhibitor-Induced Reduction of Urinary Albumin Excretion in Non-diabetic Patients. *Hypertension* 2006; **48**: 870-876 [DOI: 10.1161/01.HYP.0000244082.26293.2d]

21 **Anantharaman R**, Bhansali A, Bhadada SK, Kohli HS, Dutta P, Walia R, Jayaprakash P, Upreti V. Anti-albuminuric efficacy of a combination of angiotensin converting enzyme inhibitor & amp; angiotensin receptor blocker in type 1 DM with nephropathy. *Indian J Med Res* 2010; **132**: 42-47 [PMID: 20693588]

22 **Vejakama P**, Thakkinstian A, Lertrattananon D, Ingsathit A, Ngarmukos C, Attia J. Reno-protective effects of renin-angiotensin system blockade in type 2 diabetic patients: a systematic review and network meta-analysis. *Diabetologia* 2012; **55**: 566-578 [PMID: 22189484 DOI: 10.1007/s00125-011-2398-8]

23 **Woo KT**, Lau YK, Wong KS, Chiang GS. ACEI/ATRA therapy decreases proteinuria by improving glomerular permselectivity in IgA nephritis. *Kidney Int* 2000; **58**: 2485-2491 [PMID: 11115082 DOI: 10.1046/j.1523-1755.2000.00432.x]

24 **Gagliardini E**, Corna D, Zoja C, Sangalli F, Carrara F, Rossi M, Conti S, Rottoli D, Longaretti L, Remuzzi A, Remuzzi G, Benigni A. Unlike each drug alone, lisinopril if combined with avosentan promotes regression of renal lesions in experimental diabetes. *Am J Physiol Renal Physiol* 2009; **297**: F1448-F1456 [PMID: 19675181 DOI: 10.1152/ajprenal.00340.2009]

25 **Choudhury I**, Jothimalar R, Patra AK. Angiotensin Converting Enzyme Gene Polymorphism and its Association with Hypertension in South Indian Population. *Indian J Clin Biochem* 2012; **27**: 265-269 [PMID: 26405385 DOI: 10.1007/s12291-012-0217-8]

26 **Kumari S**, Sharma N, Thakur S, Mondal PR, Saraswathy KN. Beneficial role of D allele in controlling ACE levels: a study among Brahmins of north India. *J Genet* 2016; **95**: 291-295 [PMID: 27350671 DOI: 10.1007/s12041-016-0649-7]

27 **Jayapalan JJ**, Muniandy S, Pheng CS. Angiotensin Converting Enzyme Insertion/Deletion Gene Polymorphism: An Observational Study among Diabetic Hypertensive Subjects in Malaysia. *Trop J Pharm Res* 2010; **9**: 431-439 [DOI: 10.4314/tjpr.v9i5.61052]

28 **Jayapalan JJ**, Muniandy S, and Chan SP. Null association between ACE gene I/D polymorphism and diabetic nephropathy among multiethnic Malaysian subjects. *Indian J Hum Genet* 2010; **16**: 2 [DOI: 10.4103/0971-6866.69351]

29 **So WY**, Ma RCW, Ozaki R, Tong PCY, Ng MCY, Ho CS, Lam CWK, Chow CC, Chan WB, Kong APS, Chan JCN. Angiotensin-converting enzyme (ACE) inhibition in type 2 diabetic patients-interaction with ACE insertion/deletion polymorphism. *Kidney Int* 2006; **69**: 1438-43 [DOI: 10.1038/sj.ki.5000097]

30 **Ha SK**, Lee SY, Park, HS, Shin JH, Kim SJ, Kim DH, Kim KR, Lee HY, Han DS. ACE DD genotype is more susceptible than ACE II and ID genotypes to the antiproteinuric effect of ACE inhibitors in patients with proteinuric non-insulin-dependent diabetes mellitus. *Nephrol Dial Trannsplant* 2000; **15**: 1617-1623 [DOI: 10.1093/ndt/15.10.1617]

31 **Scharplatz M**, Puhan MA, Steurer J, Perna A, Bachmann LM. Does the Angiotensin-converting enzyme (ACE) gene insertion/deletion polymorphism modify the response to ACE inhibitor therapy?--A systematic review. *Curr Control Trials Cardiovasc Med* 2005; **6**: 16 [PMID: 16242049 DOI: 10.1186/1468-6708-6-16]

32 **Nakayama Y**, Nonoguchi H, Kohda Y, Inoue H, Memetimin H, Izumi Y, Tomita K. Different Mechanisms for the Progression of CKD with ACE Gene Polymorphisms. *Nephron Clin Pract* 2009; **111**: c240-c246 [PMID: 19293592 DOI: 10.1159/000209150]

33 **Penno G**, Chaturvedi N, Talmud PJ, Cotroneo P, Manto A, Nannipieri M, Luong LA, Fuller JH. Effect of angiotensin-converting enzyme (ACE) gene polymorphism on progression of renal disease and the influence of ACE inhibition in IDDM patients: findings from the EUCLID Randomized Controlled Trial. EURODIAB Controlled Trial of Lisinopril in IDDM. *Diabetes* 1998; **47**: 1507-1511 [PMID: 9726242 DOI: 10.2337/diabetes.47.9.1507]

34 **Jacobsen P**, Rossing K, Rossing P, Tarnow L, Mallet C, Poirier O, Cambien F, Parving HH. Angiotensin converting enzyme gene polymorphism and ACE inhibition in diabetic nephropathy. *Kidney Int* 1998; **53**: 1002-1006 [PMID: 9551410 DOI: 10.1111/j.1523-1755.1998.00847.x]

35 **Mtiraoui N**, Ezzidi I, Turki A, Chaieb M, Mahjoub T, Almawi WY. Renin-angiotensin-aldosterone system genotypes and haplotypes affect the susceptibility to nephropathy in type 2 diabetes patients. *J Renin Angiotensin Aldosterone Syst* 2011; **12**: 572-580 [PMID: 21421655 DOI: 10.1177/1470320310396542]

36 **Freire MB**, Ji L, Onuma T, Orban T, Warram JH, Krolewski AS. Gender-specific association of M235T polymorphism in angiotensinogen gene and diabetic nephropathy in NIDDM. *Hypertension* 1998; **31**: 896-899 [PMID: 9535411 DOI: 10.1161/01.HYP.31.4.896]

37 **Eroglu Z**, Cetinkalp S, Erdogan M, Kosova B, Karadeniz M, Kutukculer A, Gunduz C, Tetik A, Topcuoglu N, Ozgen AG, Tuzun M. Association of the angiotensinogen M235T and angiotensin-converting enzyme insertion/deletion gene polymorphisms in Turkish type 2 diabetic patients with and without nephropathy. *J Diabetes Complications* 2006; **22**: 186-190 [PMID: 18413162 DOI: 10.1016/j.jdiacomp.2006.12.004]

38 **Tarnow L**, Cambien F, Rossing P, Nielsen FS, Hansen BV, Ricard S, Poirier O, Parving HH. Angiotensinogen gene polymorphisms in IDDM patients with diabetic nephropathy. *Diabetes* 1996; **45**: 367-369 [PMID: 8593944 DOI: 10.2337/diabetes.45.3.367]

39 **Fradin S**, Goulet-Salmon B, Chantepie M, Grandhomme F, Morello R, Jauzac P, Reznik Y. Relationship between polymorphisms in the renin-angiotensin system and nephropathy in type 2 diabetic patients. *Diabetes Metab* 2002; **28**: 27-32 [PMID: 11938025]

40 **Hadjadj S**, Belloum R, Bouhanick B, Gallois Y, Guilloteau G, Chatellier G, Alhenc-Gelas F, Marre M. Prognostic value of angiotensin-I converting enzyme I/D polymorphism for nephropathy in type 1 diabetes mellitus: a prospective study. *J Am Soc Nephrol* 2001; **12**: 541-549 [PMID: 11181802]

41 **Narita I**, Goto S, Saito N, Song J, Omori K, Kondo D, Sakatsume M, Gejyo F. Angiotensinogen gene variation and renoprotective efficacy of renin-angiotensin system blockade in IgA nephropathy. *Kidney Int* 2003; **64**: 1050-1058 [PMID: 12911556 DOI: 10.1046/j.1523-1755.2003.00187.x]

P- Reviewer: Kumaresan R, Shukla R, Wada J **S- Editor**: Qiu S
L- Editor: Wang TQ **E- Editor**: Lu YJ

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com