ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2015 August 10; 6(9): 1082-1121

Published by Baishideng Publishing Group Inc

World Journal of Diabetes

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

Editorial Board

2011-2015

The *World Journal of Diabetes* Editorial Board now consists of 712 members, representing a team of worldwide experts in diabetes mellitus. They are from 56 countries, including Argentina (2), Australia (27), Austria (11), Belgium (5), Brazil (13), Canada (25), Chile (3), China (40), Cuba (1), Czech Republic (3), Denmark (16), Egypt (3), Finland (5), France (12), Germany (27), Greece (17), Hungary (4), India (28), Iran (8), Iraq (2), Ireland (3), Israel (10), Italy (56), Japan (30), Jordan (1), Kuwait (3), Lebanon (1), Malaysia (1), Malta (1), Mexico (4), Netherlands (9), New Zealand (3), Nigeria (2), Norway (2), Oman (3), Pakistan (2), Poland (7), Portugal (1), Qatar (1), Romania (2), Saudi Arabia (1), Singapore (4), Slovakia (1), South Africa (1), South Korea (15), Spain (24), Sweden (5), Switzerland (4), Thailand (4), Tunisia (1), Turkey (13), United Arab Emirates (3), United Kingdom (27), United States (213), Venezuela (1), and Yemen (1).

Subrata Chakrabarti, *London*
David Cherney, *Ontario*
Mervyn Deitel, *Toronto*
Jean-Pierre Després, *Quebec*
David Joseph Hill, *London*
Tian-Ru Jin, *Toronto*
Arulmozhi D Kandasamy, *Edmonton*
Jennifer L Kuk, *Toronto*
Ismail Laher, *Vancouver*
Roger S McIntyre, *Toronto*
David Meyre, *Ontario*
Joseph Fomusi Ndisang, *Saskatoon*
Raj Padwal, *Alberta*
Ciriaco A Piccirillo, *Montreal*
Remi Rabasa-Lhoret, *Montreal*
AM James Shapiro, *Edmonton*
Guang Sun, *St. John's*
Valerie Taylor, *Hamilton*
Cory Toth, *Calgary*
André Tremblay, *Montréal*
Vincent C Woo, *Winnipeg*
James Roscoe Wright, *Calgary*
Xi-Long Zheng, *Calgary*

Chile

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

China

Pang-Zeng Chang, *Qingdao*
Jie Chen, *Nanjing*
Bernard Man Yung Cheung, *Hong Kong*
William Chi-shing 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 Ching Wan Ma, *Hong Kong*
Jin-Sheng Qi, *Shijiazhuang*
Wing Yee So, *Hong Kong*
Cheuk Chun Szeto, *Hong Kong*
Kathryn Tan, *Hong Kong*
Cheng-Ming Wang, *Yangzhou*
Cong-Yi Wang, *Wuhan*
Yu Wang, *Hong Kong*
Guang-Da Xiang, *Wuhan*
Bao-Feng Yang, *Harbin*
Shu-Yu Yang, *Fujian*
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

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

Denmark

Charlotte Brøns, *Gentofte*
Jens Sandahl Christiansen, *Arhus*
Flemming Dela, *Copenhagen*
Kristine Færch, *Gentofte*
Erik L Grove, *Aarhus*
Louise Groth Grunnet, *Gentofte*
R Scott Heller, *Gentofte*
Kurt Højlund, *Odense C*
Filip K Knop, *Hellerup*
Helle Markholst, *Måløv*
Jens D Mikkelsen, *Copenhagen*
Ole Hartvig Mortensen, *Copenhagen*
Oluf Pedersen, *Copenhagen*
Esben Thyssen Vestergaard, *Aarhus*
Milan Zdravkovic, *Søborg*

Egypt

Mamdouh Moawad Ali Hssan, *Cairo*
Moshira Abdel Hakim Rateb, *Cairo*
Mona Farag Schaalan, *Cairo*

Finland

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

France

Jean Claude Ansquer, *Dijon*
Bertrand Cariou, *Nantes*
Sylvie Dejager, *Rueil-Malmaison*
Naim Akhtar Khan, *Dijon*
Jean-Philippe Lavigne, *Nîmes*
Michel Marre, *Paris*
Marie-Claude Morice, *Massy*
Riccardo Perfetti, *Paris*
Gérard Said, *Paris*
Sophie Visvikis Siest, *Nancy*
Dominique Simon, *Paris*
Didier Vieau, *Villeneuve d'Ascq*

Germany

Ioanna Gouni Berthold, *Cologne*
Christa Buechler, *Regensburg*
Roland Büttner, *Heidelberg*
Michael Froehner, *Dresden*
Hammes Hans-Peter, *Mannheim*
Nadj Herbach, *Munich*
Andrea Icks, *Düsseldorf*
Thomas Jax, *Neuss*
Ulrich Arthur Julius, *Dresden*
Michael Kluge, *Munich*
Florian Lang, *Tuebingen*
Matthias Laudes, *Köln*
Ralf Lobmann, *Stuttgart*
Rafael T Mikolajczyk, *Bremen*
Andreas Stefan Mueller, *Halle (Saale)*
Karsten Müssig, *Tübingen*
Nahid Parvizi, *Neustadt am Rübenberge*
Thomas Peter Reinehr, *Datteln*
Michael Ristow, *Jena*
Sven Schinner, *Duesseldorf*
Peter Egbert Hermann Schwarz, *Dresden*
Konstantinos Stellos, *Tubingen*
Ovidiu Alin Stirban, *Bad Oeynhausen*
Diego J Walther, *Berlin*
Silvia Anette 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, *Attiki*
Nikolaos Papanas, *Alexandroupolis*
Dimitrios Papazoglou, *Alexandroupolis*
Sokratis Pastromas, *Athens*
Melpomeni Peppa, *Athens*
Christina Piperi, *Goudi*
Nicholas K Tentolouris, *Athens*
Konstantinos A Toulis, *Salonika*
Apostolos Tsapas, *Thessaloniki*
Konstantinos Tziomalos, *Thessaloniki*
Elias Zintzaras, *Thessaly*

Hungary

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

India

Deepak Narayan Amrapurkar, *Mumbai*
C V Anuradha, *Tamil Nadu*
Sarika Arora, *New Delhi*
Pitchai Balakumar, *Sivakasi*
Muthuswamy Balasubramanyam, *Chennai*
Subhabrata Chakrabarti, *Hyderabad*
Abhay Sankar Chakraborti, *Kolkata*
Tapan K Chaudhuri, *New Delhi*
Kanwaljit Chopra, *Chandigarh*
Malabika Datta, *Delhi*
Debidas Ghosh, *West Bengal*
Ravinder Goswami, *New Delhi*
Pappachan M Joseph, *Kerala*
Jothydev Kesavadev, *Kerala*
KVS Hari Kumar, *Lucknow*
Anoop Misra, *New Delhi*
Analava Mitra, *Kharagpur*
Viswanathan Mohan, *Chennai*
S P Murthy, *Bangalore*
Pallavi Panchu, *Guntur*
Usharani Pingali, *Hyderabad*
Ambady Ramachandran, *Egmore Chennai*
Vadde Ramakrishna, *Kadapa*

Geetha Vani Rayasam, *Haryana*
Rajat Sandhir, *Chandigarh*
Manju Sharma, *New Delhi*
Suman Bala Sharma, *Delhi*
Tarun Sharma, *Chennai*

Iran

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

Iraq

Saad Abdul-Rahman Hussain, *Baghdad*
Abbas Ali Mansour, *Basrah*

Ireland

Amar Agha, *Dublin*
Mark Philip Hehir, *Dublin*
Gerald H Tomkin, *Dublin*

Israel

Michael Aviram, *Haifa*
Gal Dubnov-Raz, *Tel Hashomer*
Shimon Efrat, *Tel Aviv*
Raymond Elias Farah, *Safed*
Oren Froy, *Rehovot*
Saher Hamed, *Haifa*
Arid Nakhoul, *Haifa*
Orit Pinhas-Hamiel, *Tel Hashomer*
Haim Werner, *Tel Aviv*
Marina Shargorodsky Zimlichman, *Holon*

Italy

Luigi Angrisani, *Napoli*
Moschetta Antonio, *Bari*
Antonio Aversa, *Rome*
Roberto Baldelli, *Rome*
Giuseppe Barbaro, *Rome*
Alessandro Bartolomucci, *Parma*
Giuseppina Basta, *Pisa*
Simona Bertoli, *Milano*
Federico Bilotta, *Rome*
Fabio Broglio, *Torino*
Francesco G Chiarelli, *Chieti*
Sergio Coccheri, *Bologna*
Massimo Collino, *Torino*
Marco Aristide Comaschi, *Genoa*
Renzo Cordera, *Genova*
Francesco Dotta, *Siena*
Gagliardini Elena, *Bergamo*
Stefano 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*
Carmelo La Rosa, *Catania*
Francesco Landi, *Rome*
Monica Rosa Loizzo, *Arcavacata Rende*
Paolo Magni, *Milano*
Mariano Malaguarnera, *Catania*
Melania Manco, *Rome*
Piero Marchetti, *Pisa*
Massimo Massi-Benedetti, *Perugia*
Antonio Nicolucci, *Imbaro*
Lucia Pacifico, *Rome*
Stefano Palomba, *Catanzaro*
Giampaolo Papi, *Carpi*
Renato Pasquali, *Bologna*
Piermarco Piatti, *Milano*
Dario Pitocco, *Rome*
Antonio E Pontiroli, *Milano*
Giulio Marchesini Reggiani, *Bologna*
Giuseppe Remuzzi, *Bergamo*
Manfredi Rizzo, *Palermo*
Raffaella Rosso, *Genoa*
Giuseppe Schillaci, *Perugia*
Leonardo A Sechi, *Sassari*
Imad Sheiban, *Torino*
Cesare R Sirtori, *Milano*
Giovanni Tarantino, *Naples*
Giovanni Targher, *Verona*
Donadon Valter, *Pordenone*
Alberto Verrotti, *Chieti*
Andrea Viggiano, *Napoli*
Gianvincenzo Zuccotti, *Milan*

Japan

Masato Asahina, *Chiba*
Takuya Awata, *Saitama*
Yuichiro Eguchi, *Saga*
Goji Hasegawa, *Kyoto*
Satoshi Inoue, *Tokyo*
Eiji Ishimura, *Osaka*
Masayuki Iwano, *Nara*
Takashi Kadowaki, *Tokyo*
Eisuke Kagawa, *Hiroshima*
Masahito Katahira, *Aichi*
Eiji 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, *Tochigi*
Takashi Togo, *Yokohama*
Jun Udagawa, *Izumo*
Yoshinari Uehara, *Fukuoka*
Takuya Watanabe, *Tokyo*
Toshihiko Yada, *Tochigi*
Tohru Yorifuji, *Osaka*

Jordan

Yousef S Khader, *Irbid*

Kuwait

Kamal AA Sulaiman Al-Shoumer, *Kuwait*
Ibrahim Fadil Benter, *Safat*
Abu Salim Mustafa, *Kuwait*

Lebanon

Ramzi F Sabra, *Beirut*

Malaysia

Mafauzy Mohamed, *Kota Bharu*

Malta

Charles Savona-Ventura, *Msida*

Mexico

Manuel González-Ortiz, *Guadalajara*
Fernando Guerrero-Romero, *Durango*
Jesus Alberto Olivares-Reyes, *Mexico City*
Rocío Salceda, *Mexico City*

Netherlands

Sander Kersten, *Wageningen*
Nanne Kleefstra, *Zwolle*
Edwin Mariman, *Maastricht*
Don Poldermans, *Rotterdam*
François Pouwer, *Tilburg*
Han Roelofsen, *Groningen*
Hendrik-Jan Schuurman, *Utrecht*
Suat Simsek, *Alkmaar*
Marcel Twickler, *Bergen op Zoom*

New Zealand

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

Nigeria

Adejuwon A Adeneye, *Lagos*
Anthonia Okeoghene Ogbera, *Lagos*

Norway

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

George Louis Bakris, *Chicago*
Alistair J Barber, *Hershey*
Daniel C Batlle, *Chicago*
David SH Bell, *Birmingham*
Rita Bortell, *Worcester*
Sebastien G Bouret, *Los Angeles*
David Lloyd Brown, *Stony Brook*
Lu Cai, *Louisville*
Jack D Caldwell, *Erie*
Anna C Calkin, *Los Angeles*
Roberto A Calle, *Groton*
R Keith Campbell, *Pullman*
Carlos Campos, *New Braunfels*
Heping Cao, *New Orleans*
Krista Casazza, *Birmingham*
Aaron Brandon Caughey, *Portland*
Eileen R Chasens, *Pittsburgh*
Munmun Chattopadhyay, *Ann Arbor*
Xiao-Li Chen, *St Paul*
Sheri Renee Colberg, *Norfolk*
Craig Ian Coleman, *Hartford*
Robert Russell Conley, *Indianapolis*
Colleen M Croniger, *Cleveland*
Doyle M Cummings, *Greenville*
William C Cushman, *Memphis*
Patricia Ann D'Amore, *Boston*
Patricia Darbishire, *West Lafayette*
Guillaume Darrasse-Jèze, *New York*
Ravi M Dasu, *Sacramento*
Michael Harvey Davidson, *Chicago*
Prakash Deedwania, *SanFrancisco*
Hong-Wen Deng, *Kansas City*
Teresa P DiLorenzo, *Bronx*
Scot E Dowd, *Lubbock*
Samuel C Durso, *Baltimore*
Krystal L Edwards, *Dallas*
Alexander M Efanov, *Indianapolis*
Azza B El-Remessy, *Augusta*
Amy Zhihong Fan, *Atlanta*
Melissa Spezia Faulkner, *Tucson*
George S Ferzli, *Staten Island*
Paolo Fiorina, *Boston*
James Edward Foley, *East Hanover*
Samuel N Forjuoh, *Temple*
Alessia Fornoni, *Miami*
Martha M Funnell, *Ann Arbor*
Trudy Gaillard, *Columbus*
Pietro Galassetti, *Irvine*
Claudia Gragnoli, *Hershey*
Jennifer B Green, *Durham*
Gary J Grover, *Piscataway*
Alok Kumar Gupta, *Baton Rouge*
Werner K Gurr, *New Haven*
Samy L Habib, *San Antonio*
Abdel Rahim Hamad, *Baltimore*
Daniel M Herron, *New York*
Tiffany Hilton, *Rochester*
Raimund Hirschberg, *Torrance*
Michael Francis Holick, *Boston*
Zhaoyong Hu, *Houston*
Rachel Mary Hudacko, *New Brunswick*
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 Evers Katholi, *Springfield*
Melina Rae 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 Laferrère, *New York*
Sang Yeoup Lee, *Mayo*
Cong-Jun Li, *Beltsville*
Ching-Shwun Lin, *San Francisco*
Julie Lin, *Boston*
Shuo Lin, *Los Angeles*
Peter Lindgren, *San Diego*
James F List, *Princeton*
Dong-Min Liu, *Blacksburg*
Zhen-Qi Liu, *Charlottesville*
George William Lyerly, *Conway*
Jian-Xing Ma, *Oklahoma City*
Rong Ma, *Fort Worth*
Xin-Laing Ma, *Philadelphia*
David Maggs, *San Diego*
Kenneth Maiese, *Detroit*
Kevin C Maki, *Glen Ellyn*
Sridhar Mani, *Bronx*
Suresh Mathews, *Auburn*
Lauraar McCabe, *East Lansing*
Sarah E Messiah, *Miami*
Thomas O Metz, *Richland*
Shannon A Miller, *Orlando*
Murielle Mimeault, *Omaha*
Raghavendra G Mirmira, *Indianapolis*
Prasun J Mishra, *Bethesda*
Reema Mody, *Grayslake*
Arshag D Mooradian, *Jacksonville*
Mohammad Reza Movahed, *Tucson*
James Mu, *Rahway*
Muraleedharan G Nair, *East Lansing*
Manuel F Navedo, *Seattle*
Charles B Nemeroff, *Atlanta*
Joshua J Neumiller, *Spokane*
Steven Nissen, *Cleveland*
Hirofumi Noguchi, *Fort Worth*
Craig Nunemake, *Charlottesville*
Patrick J O'Connor, *Minneapolis*
Erin St Onge, *Apopka*
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*
Walter J Pories, *Greenville*
Parviz M Pour, *Omaha*
Wei Qiao Qiu, *Boston*
Teresa Quattrin, *Buffalo*
Cristina Rabadán-Diehl, *Bethesda*
Rajendra S Raghow, *Memphis*
Swapnil Rajpathak, *Bronx*
Armin Rashidi, *Norfolk*
Mohammed S Razzaque, *Boston*
Beverly A S Reyes, *Philadelphia*
David Rodbard, *Potomac*
Helena W Rodbard, *Rockville*
June Hart Romeo, *Cleveland*
Raul J Rosenthal, *Fort Lauderdale*
Juan M Saavedra, *Bethesda*
Stephen W Schaffer, *Mobile*
Frank AJL Scheer, *Boston*
Richard E Scranton, *Tiverton*
Vallabh (Raj) O Shah, *Albuquerque*
Aziz Shaibani, *Houston*
Jin-Xiong She, *Augusta*
Guo-Ping Shi, *Boston*
Carol Ann Shively, *Winston-Salem*
Anders AF Sima, *Detroit*
Pramil N Singh, *Loma Linda*
Rajan Singh, *Los Angeles*
Jay S Skyler, *Miami*
Dawn Smiley, *Atlanta*
Matthew D Solomon, *Stanford*
Mark A Sperling, *Pittsburgh*
Rakesh K Srivastava, *Tyler*
Bangyan Stiles, *Los Angeles*
Yu-Xiang Sun, *Houston*
Salim Surani, *Corpus Christi*
Arthur L M Swislocki, *Martinez*
Ya-Xiong Tao, *Auburn*
John A Tayek, *Torrance*
John Gaylord Teeter, *New Haven*
Carlos Marcelo Telleria, *Vermillion*
Christopher Gordon Thanos, *Providence*
Ronald G Tilton, *Galveston*
Serena Tonstad, *Loma Linda*
Michael Lawrence Traub, *Staten Island*
Guillermo E Umpierrez, *Atlanta*
Margrit Urbanek, *Chicago*
Vladimir N Uversky, *Indianapolis*
Gabriel I Uwaifo, *Baton Rouge*
Volker Vallon, *San Diego*
Shambhu D Varma, *Baltimore*
Maria Virella, *Charleston*
Hong-Jun Wang, *Boston*
Mark E Williams, *Boston*
Nathan D Wong, *Irvine*
Guangyu 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*
Yisang Yoon, *Rochester*
Yi-Hao Yu, *New York*
Kevin CJ Yuen, *Portland*
Ian Stuart Zagon, *Hershey*
Robert Yuk-Lung Zee, *Boston*
Cui-Lin Zhang, *Rockville*
James Xuejie Zhang, *Richmond*
Sarah Zhang, *Oklahoma*
Guixiang Zhao, *Atlanta*
Yang Zhao, *Indianapolis*
Ming-Hui Zou, *Oklahoma City*

Venezuela

Fuad Lechin, *Caracas*

Yemen

Khaled Abdul-Aziz Ahmed, *Ibb*

World Journal of Diabetes

Contents

Biweekly Volume 6 Number 9 August 10, 2015

World Journal of Diabetes
Volume 6 Number 9 August 10, 2015

Contents

NAME OF JOURNAL
World Journal of Diabetes

ISSN
ISSN 1948-9358 (online)

LAUNCH DATE
March 15, 2010

FREQUENCY
Biweekly

Xiu-Xia Song, Vice Director
World Journal of Diabetes
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381891
Fax: +86-10-85381893
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: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

PUBLICATION DATE
August 10, 2015

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v6.i9.1082

World J Diabetes 2015 August 10; 6(9): 1082-1091
ISSN 1948-9358 (online)

EDITORIAL

Glucose control in critical care

Jeremy Clain, Kannan Ramar, Salim R Surani

Jeremy Clain, College of Medicine, Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN 55905, United States

Kannan Ramar, Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN 55905, United States

Salim R Surani, Division of Pulmonary, Critical Care and Sleep Medicine, Texas AM University, Aransas Pass, TX 78336, United States

Author contributions: Clain J, Ramar K and Surani SR contributed to this paper.

Conflict-of-interest statement: None of the authors have any conflict of interest.

Correspondence to: Salim R Surani, MD, MPH, MSHM, FACP, FCCP, Associate Professor, Division of Pulmonary, Critical Care and Sleep Medicine, Texas AM University, 1177 West Wheeler Ave, Suite 1, Aransas Pass, TX 78336, United States. srsurani@hotmail.com
Telephone: +1-361-8857722
Fax: +1-361-8507563

Received: January 29, 2015
Peer-review started: February 2, 2015
First decision: June 18, 2015
Revised: July 14, 2015
Accepted: July 29, 2015
Article in press: August 3, 2015
Published online: August 10, 2015

Abstract

Glycemic control among critically-ill patients has been a topic of considerable attention for the past 15 years. An initial focus on the potentially deleterious effects of hyperglycemia led to a series of investigations regarding intensive insulin therapy strategies that targeted tight glycemic control. As knowledge accumulated, the pursuit of tight glycemic control among critically-ill patients came to be seen as counterproductive, and moderate glycemic control came to dominate as the standard practice in intensive care units. In recent years, there has been increased focus on the importance of hypoglycemic episodes, glycemic variability, and premorbid diabetic status as factors that contribute to outcomes among critically-ill patients. This review provides a survey of key studies on glucose control in critical care, and aims to deliver perspective regarding glycemic management among critically-ill patients.

Key words: Glycemic control; Critical care; Blood sugar in intensive care unit; Diabetes in intensive care unit; Glycemic control

Core tip: Glucose control among critically-ill patients has been an area of active research and considerable controversy in the past 15 years. This review provides a practical guide to the evidence, with a survey of the key studies that have informed current perspectives and clinical guidelines related to glycemic management among the critically ill. The article shows why initial enthusiasm for tight glycemic control waned as evidence accumulated favoring more modest glucose goals. The article also summarizes recent work investigating the importance of hypoglycemic episodes, glycemic variability, and premorbid diabetic status on morbidity and mortality in the intensive care unit.

Clain J, Ramar K, Surani SR. Glucose control in critical care. *World J Diabetes* 2015; 6(9): 1082-1091 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i9/1082.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i9.1082

INTRODUCTION

In 2001, van den Berghe *et al*[1] reported results from a single-center, prospective, randomized controlled trial in Leuven, Belgium, and changed the way that blood glucose was managed in intensive care units (ICUs) throughout the world. Prior to the publication of this first Leuven study, glycemic control among critically-ill patients received scant attention, either at the bedside or in academic journals. The overwhelmingly favorable results of the study - which, among critically-ill surgical patients, found a remarkable mortality benefit from the use of intensive insulin therapy targeting normoglycemia - sparked strong interest in glycemic management in the ICU. Intensive insulin therapy quickly became the standard of care in both medical and surgical ICUs. However, as has been the experience in many facets of critical care, promising initial single-center results were not duplicated in subsequent trials. The publication of the NICE-SUGAR trial in 2009, which reported that intensive insulin therapy may actually result in increased mortality among critically-ill patients, served as a major bookend to the era of tight glycemic control as a pillar of ICU management[2].

Nonetheless, interest in defining optimal glycemic control among critically-ill patients has continued. In the years that have followed the publication of the NICE-SUGAR trial, investigations have focused on establishing which factors of glycemic control and dysregulation most affect patient outcomes in the ICU. It has been increasingly recognized that hypoglycemia, glycemic variability, and premorbid diabetic status are all important considerations to be taken into account when approaching the glycemic management of a critically-ill patient.

This review aims to provide a survey of the key studies that have informed the changes in thinking in the past 15 years as regards glucose control in critical care. It explores the basis of the initial enthusiasm for, and subsequent skepticism of, intensive insulin therapy in the ICU. It also aims to provide perspective regarding major issues of glycemic management among critically-ill patients: hyperglycemia, hypoglycemia, glycemic variability, and premorbid diabetic status.

HYPERGLYCEMIA

Elevated blood sugar levels are commonly seen among critically ill patients, including those without a known history of diabetes. There are many reasons why patients undergoing treatment for critical illness develop hyperglycemia, and these reasons include both effects of endogenous stress responses and byproducts of medical interventions. Inflammatory cytokines and stress hormones, including cortisol and epinephrine, serve to inhibit insulin release and promote insulin resistance, thereby naturally increasing blood glucose levels by stimulating gluconeogenesis and glycogenolysis while impeding glucose uptake by peripheral tissues[3,4]. Many medical therapies further promote hyperglycemia, including the administration of exogenous catecholamines and corticosteroids, the infusion of dextrose for the purpose of suspending intravenous medications or providing parenteral nutrition, and even bedrest, which in and of itself may serve to impair glucose uptake in skeletal muscles[5,6].

Prior to the publication of the first Leuven trial[1], many practitioners viewed moderately severe hyperglycemia among critically ill patients to be either an epiphenomenon or an adaptive response, not warranting significant concern or intervention[7]. However, as observational studies accumulated linking hyperglycemia to negative in-hospital patient outcomes, this permissive attitude began to change[8-11]. Hyperglycemia was coming to be seen as complication worthy of physician attention. For example, a retrospective study of 1826 patients admitted to a mixed ICU in Stamford, Connecticut serving medical, surgical, and coronary patients reported reduced survival among those with elevated mean blood glucose levels, with a stepwise effect resulting in higher mortality as mean blood glucose levels rose[8]. Compared to patients who survived to hospital discharge, those who died had higher initial (175 mg/dL *vs* 151 mg/dL), mean (172 mg/dL *vs* 138 mg/dL), and maximum (258 mg/dL *vs* 177 mg/dL) blood glucose levels. In-hospital mortality was 9.6% among those with a mean blood glucose of 80-99 mg/dL, 29.4% among those with a mean blood glucose of 180-199 mg/dL, and 42.5% among those with a mean blood glucose greater than 300 mg/dL.

Observations such as these raised concern that acute hyperglycemia was itself contributing to poor outcomes, potentially by leaving affected patients susceptible to at least some of the consequences that have long been observed among chronic diabetics, including high infection rates, poor wound healing, and polyneuropathy[1,5]. Laboratory studies have also raised concerns about the possible deleterious effects of acute hyperglycemia, as hyperglycemia has been shown to cause injury to a variety of cell types that exhibit insulin-independent glucose uptake, including endothelial cells, hepatocytes, and renal tubular cells[12-16].

The repeated observation that hyperglycemia is associated with worse outcomes among critically ill patients, together with the theoretical harms of acutely elevated blood glucose levels, represents the basis for focusing on glycemic control in the intensive care setting. However, the possibility remains that elevated blood glucose levels are actually beneficial to the critically ill individual, and that stress hyperglycemia is an appropriate and adaptive response to life-threatening illness, as no randomized trial investigating glycemic control has studied the effect of truly permissive hyperglycemia[17]. Potential benefits of hyperglycemia in the critically ill individual include promotion of glucose delivery in the face of ischemic insults (down an enhanced glucose diffusion gradient), with insulin resistance favoring redistribution of available glucose

stores toward cells of the immune and nervous systems, and away from peripheral tissues[17]. Recent observational studies have provided some support for this view, reasserting the possibility that hyperglycemia is simply a marker of illness severity. For example, a recent retrospective study of 7925 consecutive critically ill patients admitted to three mixed ICUs in Australia showed that while hyperglycemia was associated with in-hospital mortality, once lactate levels were considered, there was no independent association between hyperglycemia and mortality[18]. This finding was consistent with a previous retrospective study, which found that among a cohort of septic nondiabetic adult patients, hyperglycemia noted on initial presentation did not increase mortality risk unless accompanied by concurrent hyperlactatemia[19]. Such observations present a useful reminder that our understanding of the effects of hyperglycemia remains incomplete.

Our ability to identify patients most likely to suffer harm from hyperglycemia also remains incomplete. Several studies have concluded that the association between hyperglycemia and in-hospital mortality is attenuated among those with pre-existing diabetes mellitus, with some even failing to demonstrate any association at all[11,20-23].

MAJOR INVESTIGATIONS OF GLYCEMIC CONTROL IN CRITICALLY ILL PATIENTS

Concern about the potentially deleterious effects of hyperglycemia in critically ill patients has motivated multiple randomized controlled trials investigating glycemic management in ICUs[1,2,24-32]. This section serves to review the major trials regarding this subject, exploring the evidence that underlay the initial enthusiasm for, and subsequent skepticism of, intensive insulin therapy for glycemic normalization among critically ill patients. Key features of the trials are summarized in Table 1.

The original Leuven study, reported by van den Berghe *et al*[1] in 2001, was the first major prospective trial to investigate the effects of tight glycemic control in critically ill patients. This was a prospective, non-blinded, randomized controlled trial of 1548 mechanically ventilated adult patients admitted to a single surgical ICU in Leuven, Belgium. A majority of the patients (63%) had undergone cardiac surgery. Prior to admission, 13% of patients had been diagnosed with diabetes mellitus, and 5% had been maintained on insulin therapy. Upon ICU admission, patients were randomly assigned to receive either "intensive" or "conventional" insulin therapy. For all patients, insulin was delivered *via* a continuous infusion, and glycemic monitoring was performed *via* measurements of whole-blood glucose of arterial blood samples, collected every one to four hours. For patients in the intensive insulin therapy group, insulin infusions were started if measures of blood glucose exceeded 110 mg/dL, and the infusions were titrated to maintain blood glucose in the range of 80 to 110 mg/dL. By contrast, for patients in the conventional therapy group, insulin infusions were only started if measures of blood glucose exceeded 215 mg/dL, and the infusions were titrated to maintain blood glucose in the range of 180 to 200 mg/dL. All patients received intravenous glucose for the first 24 h of ICU admission, after which feeding continued *via* total parenteral, total enteral, or combined enteral and parenteral nutrition. All patients reverted to conventional blood glucose management upon discharge from the ICU. During their ICU stays, 98.7% of patients in the intensive insulin therapy group required insulin infusions, and the targeted blood glucose level was achieved, with a mean blood glucose of 103 mg/dL. Among patients in the conventional insulin therapy group, only 39.2% required insulin infusions, and the mean blood glucose was 153 mg/dL. The results of the study strongly favored the intensive insulin therapy group, with observed benefits in terms of both morbidity and mortality. In-ICU mortality was 4.6% in the intensive insulin therapy group compared to 8.0% in the conventional insulin therapy group ($P < 0.04$), and the survival benefit persisted to hospital discharge, with an absolute risk reduction of in-hospital mortality of 3.7% (7.2% *vs* 10.9%; $P = 0.01$), largely due to a reduction in deaths attributed to sepsis. Compared to patients in the conventional insulin therapy group, those receiving intensive insulin therapy also experienced reduced rates of renal replacement therapy, prolonged mechanical ventilation, and extended ICU stays. The overwhelmingly positive results from the first Leuven study were in many ways practice-changing, and it informed investigations into glycemic management of critically ill patients for the ensuing decade, and beyond.

The next major prospective trial came from the same group in Belgium, and was again a single-ICU study[24]. In this second Leuven study, 1200 adult patients admitted to a medical ICU were studied. The study included only patients who were unable to take oral nutrition upon ICU admission, and who were anticipated to require at least 3 d of intensive care. Patients were randomized to intensive *vs* conventional insulin therapy groups, with stratification according to diagnostic categories. Thresholds for initiation of insulin therapy and target blood glucose levels for the two groups were identical to what had been used in the first Leuven study[24]. In stark contrast to the findings of the previous trial, the second Leuven study showed no overall mortality benefit to intensive insulin therapy, as both ICU and in-hospital mortality rates were similar among patients in the intensive and conventional insulin therapy groups. However, the authors reported a statistical difference in in-hospital mortality among the subset of patients who actually received at least 3 d of ICU care, as had been intended at the time of their inclusion in the study. Among this subset of 767 patients who stayed in the ICU for at least 3 d (of whom 386 received intensive insulin therapy and 381 received conventional insulin therapy), in-hospital mortality was

Table 1 Summary of major randomized controlled trials investigating the use of intensive *vs* conventional insulin therapy in critically-ill patients

Trial	Study population	Number of patients enrolled	Target blood glucose in the intensive insulin therapy group (mg/dL)	Target blood glucose in the conventional insulin therapy group (mg/dL)	Key mortality findings	Key morbidity findings
First Leuven Trial, 2001[1]	Single-center; surgical ICU	1548	80-110	180-200	Reduced ICU and in-hospital mortality in the intensive insulin therapy group	Reduced incidence of bloodstream infection, acute renal failure, red cell transfusion, and critical-illness neuropathy in the intensive insulin therapy group. Increased occurrence of severe hypoglycemia in the intensive insulin therapy group
Second Leuven Trial, 2006[24]	Single-center; medical ICU	1200	80-110	180-200	No mortality difference	Reduced incidence of newly acquired kidney injury, reduced duration of mechanical ventilation, and reduced lengths of ICU and hospital stays in the intensive insulin therapy group. Increased occurrence of hypoglycemia in the intensive insulin therapy group
Arabi *et al*[25], 2008	Single-center; mixed ICU, including medical, surgical, and trauma patients	523	80-110	180-200	No mortality difference	Increased occurrence of hypoglycemia in the intensive insulin therapy group
Brunkhorst *et al*[26], 2008	Multicenter; mixed ICUs; all patients with severe sepsis or septic shock	537	80-110	180-200	No mortality difference	No difference in the mean score for organ failure. Increased occurrence of severe hypoglycemia in the intensive insulin therapy group
De La Rosa Gdel *et al*[27], 2008	Single center; mixed ICU, including medical, surgical, and trauma patients	504	80-110	180-200	No mortality difference	Increased occurrence of severe hypoglycemia in the intensive insulin therapy group
Preiser *et al*[28], 2009	Multicenter; medical and surgical ICU patients	1078	79-110	140-180	No mortality difference	Increased occurrence of severe hypoglycemia in the intensive insulin therapy group
NICE-SUGAR Trial, 2009[2]	Multicenter; medical and surgical ICU patients	6104	81-108	144-180	Increased 90-d mortality in the intensive insulin therapy group	Similar between-group markers of morbidity, with the exception of an increased occurrence of severe hypoglycemia in the intensive insulin therapy group
Annane *et al*[29], 2010	Multicenter; all patients with septic shock	509	80-110	180-200	No mortality difference	Increased occurrence of severe hypoglycemia in the intensive insulin therapy group
Coester *et al*[30], 2010	Single center; all patients with severe traumatic brain injury	88	80-110	< 180	No mortality difference	No difference in neurologic outcomes. Increased occurrence of hypoglycemia in the intensive insulin therapy group
Green *et al*[31], 2010	Single center; mechanically-ventilated neurologic patients	81	80-110	⩽ 150	No mortality difference	No difference in neurologic function at 90 d. Increased occurrence of hypoglycemia and severe hypoglycemia in the intensive insulin therapy group
Macrae *et al*[32], 2014	Multicenter; medical and surgical pediatric patients	1369	72-126	180-216	No mortality difference	No difference in ventilator-free survival. Increased occurrence of severe hypoglycemia in the intensive insulin therapy group

ICU: Intensive care unit.

43.0% in the intensive therapy group, compared to 52.5% in the conventional therapy group (P = 0.009). While an interesting finding, this subset analysis suffered from a lack of real-world applicability (even the authors were unable to accurately predict which patients would require extended ICU stays) and a loss of balanced diagnostic categorization (likely biasing the results). While no mortality benefit to intensive insulin therapy was identified, secondary analyses of patient morbidity found reduced rates of acquired kidney injury, reduced durations of mechanical ventilation, and reduced lengths of ICU and hospital stay among patients in the intensive insulin therapy group compared to those in the conventional insulin therapy group.

The mortality benefits realized in the first Leuven study and the morbidity benefits realized in the second sustained considerable enthusiasm for tight glycemic control in critically ill patients for the next several years, with widespread adoption of intensive insulin protocols in medical and surgical ICUs, despite occasional voices urging caution[33,34]. However, a series of studies published in 2008 and 2009, culminating with the NICE-SUGAR trial, severely tempered this enthusiasm[2,25-28]. The first of these trials, reported by Brunkhorst *et al*[26], involved patients with severe sepsis or septic shock admitted to multidisciplinary ICUs in 18 academic tertiary hospitals in Germany. This was a two-by-two factorial trial, and patients were randomized to receive either intensive or conventional insulin therapy for glycemic control (with protocols similar to those used in the two Leuven studies[1,24]) and either hydroxyethyl starch or modified Ringer's lactate for fluid resuscitation. The use of intensive insulin therapy was terminated after the first safety analysis, due to a nearly six-fold increased frequency of hypoglycemia in the intensive insulin group, including a high proportion of severe hypoglycemic events that were classified as life-threatening and requiring prolonged hospitalization. Among the patients studied, there was no documented benefit to intensive insulin therapy, as there were no statistical differences in rates of mortality, rates of acute renal failure or renal replacement therapy, use of vasopressor medications, number of ventilator-free days, or length of ICU stay.

Several subsequent studies conducted in a variety of settings similarly failed to demonstrate clear benefits to tight glycemic control in critically ill patients, but consistently highlighted an increased risk of hypoglycemia among patients treated with intensive insulin protocols[2,25,27,28]. Arabi *et al*[25] reported a prospective trial wherein they randomized 523 medical, surgical, and trauma patients admitted to a single ICU in Riyadh, Saudi Arabia to intensive or conventional insulin therapy, and found no between-group differences in mortality, ICU or hospital lengths of stay, rates of renal replacement therapy, durations of mechanical ventilation, or frequencies of infectious complications, but patients in the intensive insulin group experienced much higher rates of hypoglycemia. Similar negative findings with respect to measures of mortality and morbidity were reported by De La Rosa Gdel *et al*[27] in their study of 504 medical, surgical, and trauma patients admitted to a single ICU in Medellin, Colombia and randomized to intensive or conventional insulin therapy, though again, rates of hypoglycemia were much higher in the intensive insulin group. A subsequent multinational trial, involving patients admitted to 21 medico-surgical ICUs in 7 countries, also failed to identify meaningful benefits to tight glycemic control[28]. This study, which again randomized patients to intensive or conventional insulin therapy, was ultimately underpowered, as it was prematurely stopped due to a high rate of unintended protocol violations. However, among the 1078 patients studied, there were no between-group differences in mortality, and the only differences in measures of morbidity were higher rates of hypoglycemia among patients in the intensive insulin therapy group and a slight reduction in vasopressor/inotrope use in the conventional insulin therapy group.

On the heels of these four consecutive negative studies[25-28], the landmark NICE-SUGAR trial was reported, which remains the most comprehensive study of glycemic control strategies among ICU patients performed to date[2]. The NICE-SUGAR study included 6104 medical and surgical patients admitted to ICUs at 42 hospitals in Australia, New Zealand, Canada, and the United States. All patients were anticipated to require at least 3 d of ICU care, were expected to be unable to eat for at least 2 d, and had an arterial line in place as part of their routine ICU management. As with previous studies, patients were randomized to intensive or conventional insulin therapy groups, but the target blood glucose range of the conventional insulin therapy group was lower than it had been in the Leuven studies[1,24], based on updated practice surveys. In the intensive insulin therapy group, the target blood glucose range was 81 to 108 mg/dL, while in the conventional insulin therapy group, the target blood glucose was 180 mg/dL or less, with insulin administration reduced and then discontinued if blood glucose levels fell below 144 mg/dL. As had been the case in Leuven studies[1,24], blood glucose monitoring was performed every one to four hours, and the use of arterial rather than capillary blood samples for this purpose was encouraged. The majority of patients in both treatment groups received insulin therapy (97.2% of those in the intensive insulin therapy group and 69.0% of those in the conventional insulin therapy group). The mean time-weighted blood glucose level in the intensive group was 115 mg/dL, while it was 144 mg/dL in the conventional group. The primary study endpoint was 90-d all-cause mortality, which was 2.6% higher in the intensive than in the conventional insulin therapy group (27.5% *vs* 24.9%, P = 0.02). Subgroup analyses suggested no differences in treatment effects for comparisons of medical and surgical patients, patients with and without preexisting diabetes, and patients with and without severe sepsis. With the exception of rates of severe hypoglycemia, markers of morbidity did not differ according to treatment groups,

as there were similar between-group ICU and hospital lengths of stay, durations of mechanical ventilation, frequencies and durations of renal replacement therapy, rates of new organ failure, and occurrences of positive blood cultures. Severe hypoglycemia (defined as a blood glucose level less than or equal to 40 mg/dL) occurred in 6.8% of the patients in the intensive insulin therapy group *vs* 0.5% of those in the conventional therapy group ($P < 0.001$).

Following the overwhelmingly negative results of the NICE-SUGAR study, Annane *et al*[29] reported on the use of intensive *vs* conventional insulin therapy in patients with septic shock being treated with corticosteroids, hypothesizing that this subset of ICU patients may benefit from intensive insulin therapy, even if a general ICU population does not. A total of 509 patients treated in 11 ICUs in France were randomized to intensive or conventional insulin therapy, according to the treatment protocols used in the first Leuven study[1]. Here again, there were no between-group differences in measures of patient mortality or morbidity, with the exception of an increased rate of severe hypoglycemia among patients in the intensive insulin therapy group. Subsequently, randomized controlled trials investigating intensive insulin therapy among mechanically ventilated neurologic patients, patients with severe traumatic brain injuries, and critically ill pediatric patients have all failed to demonstrate a clinical benefit to tight glycemic control[30-32].

In summary, following the publication of the two single-center Leuven studies[1,24], the preponderance of evidence has strongly indicated that the use of intensive insulin treatment with the goal of tight glycemic management in critically-ill patients at best provides no benefit over moderate or lax glycemic control, and at worst results in markedly increased rates of severe hypoglycemia and possibly even increased mortality[2,25-29].

HYPOGLYCEMIA

As clinicians and investigators have grappled with the results of the NICE-SUGAR trial and of other negative studies regarding the use of intensive insulin therapy in critically-ill patients[2,25-32], several potential explanations have been proposed to account for the lack of demonstrable benefit for tight glucose control. The proposed explanations have targeted either the rationale for intensive insulin therapy (positing that hyperglycemia may be beneficial, or that exogenous insulin may be harmful), or the execution of the strategy (suggesting that the labor-intensive focus on tight glycemic control distracts from other considerations, or that the benefits of normoglycemia have been obscured by an inability to avoid hypoglycemia)[4,35]. This final consideration-that hypoglycemic complications negate the potential benefits of tight glycemic control-has gained widespread acceptance, and has important implications for future study of glycemic management among critically-ill patients. Hypoglycemia has been a commonly-reported occurrence among the patients treated with intensive insulin therapy in major trials, and severe hypoglycemia (defined as a blood glucose level less than 40 mg/dL) has occurred in up to 28% of these patients[4]. It was not initially clear whether the increased rate of hypoglycemia experienced among patients treated with a tight glycemic control strategy was problematic. In the first Leuven study, severe hypoglycemia was reported to have occurred 6.6-fold more commonly among patients in the intensive insulin therapy group, but no clinically-significant outcomes were associated with its occurrence in any of the patients, and the issue of hypoglycemia was not addressed in the manuscript's discussion[1].

By the time the NICE-SUGAR trial was reported, the frequency of hypoglycemic episodes among patients treated with intensive insulin regimens had become a significant concern. It was recognized that hypoglycemia could theoretically be harmful to patients by means of a number of different mechanisms, including irreversible neuronal damage, autonomic instability, cardiac arrhythmia, and alteration of inflammatory responses[36,37]. The relationship between hypoglycemia and mortality was examined in a post-hoc analysis of the NICE-SUGAR trial[37]. For the purpose of this analysis, severe hypoglycemia was defined as a recorded blood glucose level of 40 mg/dL or less, while moderate hypoglycemia was defined as a recorded blood glucose level in the range of 41 to 70 mg/dL. Among the 6026 patients analyzed, severe hypoglycemia occurred in 3.7% of individuals, while moderate hypoglycemia occurred in an additional 45.0%. Hypoglycemic episodes were much more common among those patients in the intensive insulin therapy group, with this group accounting for 93.3% of severe hypoglycemia and 82.4% of moderate hypoglycemia. The occurrence of hypoglycemia was strongly associated with an increased risk of death, with moderate hypoglycemia associated with a 40% increase in adjusted mortality risk, and severe hypoglycemia associated with a doubling of this risk. While these data do not prove a causal relationship between hypoglycemia and mortality, they do support the possibility that it was the increased frequency of iatrogenic hypoglycemic episodes that accounted in some measure for the excess mortality observed among patients treated with intensive insulin therapy in the NICE-SUGAR trial.

This possibility has been supported by other retrospective studies investigating the relationship between hypoglycemic episodes and mortality among ICU patients. In a review of 4946 patients admitted to two ICUs in Australia, Egi *et al*[38] found that 22.4% of patients experienced at least one episode of hypoglycemia, defined as recorded blood glucose of less than 82 mg/dL. The patients were analyzed in six bands, according to the level of their lowest recorded blood glucose, and it was shown that the severity of hypoglycemia was independently associated with in-hospital mortality. In a separate single-center review of 5365 consecutive patients admitted to a mixed medical-

surgical ICU, the occurrence of even one episode of severe hypoglycemia was seen to be independently associated with mortality, both by case-control and by multivariable logistic regression analyses[39].

To a significant extent, a desire to avoid inducing hypoglycemia has motivated the move away from treating ICU patients with intensive insulin protocols[40]. It should be noted that the focus on avoiding hypoglycemia leaves the door open to future reconsideration of the benefits of tight glycemic control. If the problem with intensive insulin therapy is mainly an inability to avoid hypoglycemic episodes, one can imagine that the development of better glucose monitoring technologies and glycemic control algorithms (if they allow for severe reductions in the incidence of hypoglycemia) could result in improved outcomes with a tight glycemic control strategy. In recent years, the development of continuous glucose monitoring systems has received significant attention along these lines, but the benefits of continuous glucose monitoring have not yet been established[41-43].

GLYCEMIC VARIABILITY

In recent years, it has increasingly been recognized that glycemic variability is a dimension of significant importance among critically-ill patients, independent of the acute highs and lows of blood glucose measurements in the ICU. The potential significance of glycemic variability among ICU patients was first raised by Egi *et al*[44], in a retrospective observational study of 7049 patients who had been admitted to four hospitals in Australia. For the purposes of this study, a patient's glycemic variability was defined as the standard deviation of the arithmetical mean of the entire set of glucose measurements during that individual's ICU stay. The authors found that glycemic variability was an independent predictor of mortality, and that the glycemic variability was actually a stronger predictor of ICU mortality than the mean glucose concentration. A subsequent single-center retrospective observational study of 3252 ICU patients in the United States confirmed and extended these findings, again demonstrating that this measure of glycemic variability was a strong independent predictor of mortality, even after excluding patients who had experienced severe hypoglycemia[45].

As glycemic variability has been further considered among ICU patients, definitions have changed. Defining glycemic variability as the standard deviation of the mean of all blood glucose measurements has fallen out of favor, as starkly different glycemic patterns can generate identical mean glucose and standard deviations[46]. Multiple other measures of glycemic variability have been described, including coefficient of variation, glycemic lability index, mean absolute glucose change, and mean amplitude of glycemic excursion[47,48]. No gold standard for measuring glycemic variability has been established, but multiple studies utilizing these more complicated metrics have confirmed that glycemic variability is independently associated with mortality among ICU patients[23,46,48,49].

Whether glycemic variability is a cause of poor patient outcomes or is simply a marker of severe illness is not known. However, several lines of evidence have suggested that glycemic variability causes oxidative stress, enhances cell apoptosis, and impairs endothelial function[45,46]. Therefore, it is plausible that glycemic variability causes harm to critically-ill patients, and that optimal glycemic control in the ICU would aim to minimize glycemic variability. As with avoiding hypoglycemia in the ICU, it is hoped that advances in glycemic monitoring and corresponding glucose control algorithms will reduce the extent of glycemic variability, but at least one early study has failed to show that existing means of continuous glucose monitoring would reduce glycemic variability[47].

PREMORBID DIABETIC STATUS

From the first Leuven study to the NICE-SUGAR trial, all of the major investigations of intensive insulin therapy in critically-ill patients utilized glycemic-control protocols that did not differentiate between diabetic and nondiabetic patients[1,2,24-28]. Similarly, recent guidelines regarding the use of insulin infusions in the ICU do not advocate altering the approach to glycemic management on the basis of patients' premorbid diabetic status[40]. However, there is growing evidence that diabetic and nondiabetic patients respond differently to dysglycemia experienced in the ICU.

Krinsley *et al*[49] performed a retrospective observational study of 44964 patients admitted to 23 ICUs in 9 countries to determine how diabetic status affected the associations of hyperglycemia, hypoglycemia, and glycemic variability with mortality. While hypoglycemia was associated with an increased risk of mortality among all patients, the diabetic status modulated the impact of both hyperglycemia and glycemic variability. In nondiabetic patients, maintenance of euglycemia was independently associated with a reduced mortality risk, but among diabetic patients, those with a mean glucose of 80 to 110 mg/dL actually had an increased risk of mortality, even when compared only to those with a mean glucose greater than 179 mg/dL. The significance of glycemic variability also seemed to differ according to diabetic status, as a high level of glycemic variability (defined as a coefficient of variability greater than 20%) was independently associated with an increased risk of mortality among nondiabetic patients, but not among those with diabetes.

Similar findings were reported in a subsequent single center retrospective observational study that analyzed glucose and outcome data from 10320 ICU patients[23]. Again, hypoglycemia was associated with mortality in both diabetic and nondiabetic patients, but outcomes associated with hyperglycemia and glycemic variability differed according to premorbid diabetic status. While hyperglycemia was associated with increased mortality

among the nondiabetic patients, no clear pattern relating elevated mean glucose levels with mortality could be found among the diabetic patients. In addition, glycemic variability (as measured by mean absolute glucose change) was only associated with increased mortality among the nondiabetic patients.

Such differences among diabetic and nondiabetic patients have raised the possibility that future glycemic control protocols for critically-ill patients will differ according to premorbid diabetic status, or other markers of insulin resistance, such as metabolic syndrome or non-alcoholic fatty liver disease[50,51]. However, further studies are needed to better define optimal glycemic management among diabetic patients in the ICU.

CONCLUSION

In the past two decades, glycemic management among critically-ill patients has been a topic of extensive study, leading to significant changes in clinical practice. Intensive insulin therapy was widely adopted following the publication of the first Leuven study[1], only to be largely abandoned as further knowledge accumulated questioning the benefits of this approach, ultimately culminating with the NICE-SUGAR trial, which found an increased risk of mortality among patients treated with tight, as compared to moderate, glucose control strategies[2]. Current guidelines regarding glycemic management of critically-ill patients advocate initiating insulin infusions for blood glucose measurements in excess of 150 mg/dL, with the goal of maintaining blood glucose less than 180 mg/dL[40]. While targeting a blood glucose level less than 180 mg/dL is now widespread (and consistent with the control group in NICE-SUGAR), it should be noted that evidence supporting this goal, as opposed to an even more permissive glycemic control strategy, is lacking.

In recent years, there has been an increased focus on the potential deleterious effects of glycemic variability, though it remains unclear how best to avoid fluctuations in blood glucose levels. In addition, there has been increasing attention given to differences among the glycemic control needs of diabetic and nondiabetic patients.

In coming years, we expect that new glucose monitoring systems will emerge, and that new strategies for maintaining euglycemia (while avoiding hypoglycemic episodes and glycemic variability) will follow. Glycemic management among critically-ill patients remains an area of unsettled medicine.

REFERENCES

1 **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]

2 **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]

3 **Lena D**, Kalfon P, Preiser JC, Ichai C. Glycemic control in the intensive care unit and during the postoperative period. *Anesthesiology* 2011; **114**: 438-444 [PMID: 21245729 DOI: 10.1097/ALN.0b013e3182078843]

4 **Kavanagh BP**, McCowen KC. Clinical practice. Glycemic control in the ICU. *N Engl J Med* 2010; **363**: 2540-2546 [PMID: 21175316 DOI: 10.1056/NEJMcp1001115]

5 **McCowen KC**, Malhotra A, Bistrian BR. Stress-induced hyperglycemia. *Crit Care Clin* 2001; **17**: 107-124 [PMID: 11219223]

6 **Stuart CA**, Shangraw RE, Prince MJ, Peters EJ, Wolfe RR. Bed-rest-induced insulin resistance occurs primarily in muscle. *Metabolism* 1988; **37**: 802-806 [PMID: 3043146]

7 **Chinsky K**. The evolving paradigm of hyperglycemia and critical illness. *Chest* 2004; **126**: 674-676 [PMID: 15364740 DOI: 10.1378/chest.126.3.674]

8 **Krinsley JS**. Association between hyperglycemia and increased hospital mortality in a heterogeneous population of critically ill patients. *Mayo Clin Proc* 2003; **78**: 1471-1478 [PMID: 14661676 DOI: 10.4065/78.12.1471]

9 **Finney SJ**, Zekveld C, Elia A, Evans TW. Glucose control and mortality in critically ill patients. *JAMA* 2003; **290**: 2041-2047 [PMID: 14559958 DOI: 10.1001/jama.290.15.2041]

10 **Whitcomb BW**, Pradhan EK, Pittas AG, Roghmann MC, Perencevich EN. Impact of admission hyperglycemia on hospital mortality in various intensive care unit populations. *Crit Care Med* 2005; **33**: 2772-2777 [PMID: 16352959]

11 **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]

12 **Langouche L**, Vanhorebeek I, Vlasselaers D, Vander Perre S, Wouters PJ, Skogstrand K, Hansen TK, Van den Berghe G. Intensive insulin therapy protects the endothelium of critically ill patients. *J Clin Invest* 2005; **115**: 2277-2286 [PMID: 16075063 DOI: 10.1172/JCI25385]

13 **Vanhorebeek I**, De Vos R, Mesotten D, Wouters PJ, De Wolf-Peeters C, Van den Berghe G. Protection of hepatocyte mitochondrial ultrastructure and function by strict blood glucose control with insulin in critically ill patients. *Lancet* 2005; **365**: 53-59 [PMID: 15639679 DOI: 10.1016/S0140-6736(04)17665-4]

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

15 **Vanhorebeek I**, Gunst J, Ellger B, Boussemaere M, Lerut E, Debaveye Y, Rabbani N, Thornalley PJ, Schetz M, Van den Berghe G. Hyperglycemic kidney damage in an animal model of prolonged critical illness. *Kidney Int* 2009; **76**: 512-520 [PMID: 19536085 DOI: 10.1038/ki.2009.217]

16 **Boonen E**, Van den Berghe G. Endocrine responses to critical illness: novel insights and therapeutic implications. *J Clin Endocrinol Metab* 2014; **99**: 1569-1582 [PMID: 24517153 DOI: 10.1210/jc.2013-4115]

17 **Marik PE**, Bellomo R. Stress hyperglycemia: an essential survival response! *Crit Care Med* 2013; **41**: e93-e94 [PMID: 23685597 DOI: 10.1097/CCM.0b013e318283d124]

18 **Kaukonen KM**, Bailey M, Egi M, Orford N, Glassford NJ, Marik PE, Bellomo R. Stress hyperlactatemia modifies the relationship between stress hyperglycemia and outcome: a retrospective observational study. *Crit Care Med* 2014; **42**: 1379-1385 [PMID: 24561567 DOI: 10.1097/CCM.0000000000000214]

19 **Green JP**, Berger T, Garg N, Horeczko T, Suarez A, Radeos MS, Hagar Y, Panacek EA. Hyperlactatemia affects the association of hyperglycemia with mortality in nondiabetic adults with sepsis. *Acad Emerg Med* 2012; **19**: 1268-1275 [PMID: 23167858 DOI: 10.1111/acem.12015]

20 **Rady MY**, Johnson DJ, Patel BM, Larson JS, Helmers RA. Influence of individual characteristics on outcome of glycemic control in intensive care unit patients with or without diabetes mellitus. *Mayo Clin Proc* 2005; **80**: 1558-1567 [PMID: 16342648 DOI: 10.4065/80.12.1558]

21 **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]

22 **Graham BB**, Keniston A, Gajic O, Trillo Alvarez CA, Medvedev S, Douglas IS. Diabetes mellitus does not adversely affect outcomes from a critical illness. *Crit Care Med* 2010; **38**: 16-24 [PMID: 19789450 DOI: 10.1097/CCM.0b013e3181b9eaa5]

23 **Sechterberger MK**, Bosman RJ, Oudemans-van Straaten HM, Siegelaar SE, Hermanides J, Hoekstra JB, De Vries JH. The effect of diabetes mellitus on the association between measures of glycaemic control and ICU mortality: a retrospective cohort study. *Crit Care* 2013; **17**: R52 [PMID: 23510051 DOI: 10.1186/cc12572]

24 **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]

25 **Arabi YM**, Dabbagh OC, Tamim HM, Al-Shimemeri AA, Memish ZA, Haddad SH, Syed SJ, Giridhar HR, Rishu AH, Al-Daker MO, Kahoul SH, Britts RJ, Sakkijha MH. Intensive versus conventional insulin therapy: a randomized controlled trial in medical and surgical critically ill patients. *Crit Care Med* 2008; **36**: 3190-3197 [PMID: 18936702 DOI: 10.1097/CCM.0b013e31818f21aa]

26 **Brunkhorst FM**, Engel C, Bloos F, Meier-Hellmann A, Ragaller M, Weiler N, Moerer O, Gruendling M, Oppert M, Grond S, Olthoff D, Jaschinski U, John S, Rossaint R, Welte T, Schaefer M, Kern P, Kuhnt E, Kiehntopf M, Hartog C, Natanson C, Loeffler M, Reinhart K. Intensive insulin therapy and pentastarch resuscitation in severe sepsis. *N Engl J Med* 2008; **358**: 125-139 [PMID: 18184958 DOI: 10.1056/NEJMoa070716]

27 **De La Rosa Gdel C**, Donado JH, Restrepo AH, Quintero AM, González LG, Saldarriaga NE, Bedoya M, Toro JM, Velásquez JB, Valencia JC, Arango CM, Aleman PH, Vasquez EM, Chavarriaga JC, Yepes A, Pulido W, Cadavid CA. Strict glycaemic control in patients hospitalised in a mixed medical and surgical intensive care unit: a randomised clinical trial. *Crit Care* 2008; **12**: R120 [PMID: 18799004 DOI: 10.1186/cc7017]

28 **Preiser JC**, Devos P, Ruiz-Santana S, Mélot C, Annane D, Groeneveld J, Iapichino G, Leverve X, Nitenberg G, Singer P, Wernerman J, Joannidis M, Stecher A, Chioléro R. A prospective randomised multi-centre controlled trial on tight glucose control by intensive insulin therapy in adult intensive care units: the Glucontrol study. *Intensive Care Med* 2009; **35**: 1738-1748 [PMID: 19636533 DOI: 10.1007/s00134-009-1585-2]

29 **Annane D**, Cariou A, Maxime V, Azoulay E, D'honneur G, Timsit JF, Cohen Y, Wolf M, Fartoukh M, Adrie C, Santré C, Bollaert PE, Mathonet A, Amathieu R, Tabah A, Clec'h C, Mayaux J, Lejeune J, Chevret S. Corticosteroid treatment and intensive insulin therapy for septic shock in adults: a randomized controlled trial. *JAMA* 2010; **303**: 341-348 [PMID: 20103758 DOI: 10.1001/jama.2010.2]

30 **Coester A**, Neumann CR, Schmidt MI. Intensive insulin therapy in severe traumatic brain injury: a randomized trial. *J Trauma* 2010; **68**: 904-911 [PMID: 20032790 DOI: 10.1097/TA.0b013e3181c9afc2]

31 **Green DM**, O'Phelan KH, Bassin SL, Chang CW, Stern TS, Asai SM. Intensive versus conventional insulin therapy in critically ill neurologic patients. *Neurocrit Care* 2010; **13**: 299-306 [PMID: 20697836 DOI: 10.1007/s12028-010-9417-3]

32 **Macrae D**, Grieve R, Allen E, Sadique Z, Morris K, Pappachan J, Parslow R, Tasker RC, Elbourne D. A randomized trial of hyperglycemic control in pediatric intensive care. *N Engl J Med* 2014; **370**: 107-118 [PMID: 24401049 DOI: 10.1056/NEJMoa1302564]

33 **Angus DC**, Abraham E. Intensive insulin therapy in critical illness. *Am J Respir Crit Care Med* 2005; **172**: 1358-1359 [PMID: 16301298 DOI: 10.1164/rccm.2508009]

34 **Bellomo R**, Egi M. Glycemic control in the intensive care unit: why we should wait for NICE-SUGAR. *Mayo Clin Proc* 2005; **80**: 1546-1548 [PMID: 16342646 DOI: 10.4065/80.12.1546]

35 **Inzucchi SE**, Siegel MD. Glucose control in the ICU--how tight is too tight? *N Engl J Med* 2009; **360**: 1346-1349 [PMID: 19318385 DOI: 10.1056/NEJMe0901507]

36 **Krinsley JS**, Keegan MT. Hypoglycemia in the critically ill: how low is too low? *Mayo Clin Proc* 2010; **85**: 215-216 [PMID: 20176927 DOI: 10.4065/mcp.2010.0010]

37 **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]

38 **Egi M**, Bellomo R, Stachowski E, French CJ, Hart GK, Taori G, Hegarty C, Bailey M. Hypoglycemia and outcome in critically ill patients. *Mayo Clin Proc* 2010; **85**: 217-224 [PMID: 20176928 DOI: 10.4065/mcp.2009.0394]

39 **Krinsley JS**, Grover A. Severe hypoglycemia in critically ill patients: risk factors and outcomes. *Crit Care Med* 2007; **35**: 2262-2267 [PMID: 17717490 DOI: 10.1097/01.CCM.0000282073.98414.4B]

40 **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]

41 **Hirsch IB**. Understanding low sugar from NICE-SUGAR. *N Engl J Med* 2012; **367**: 1150-1152 [PMID: 22992080 DOI: 10.1056/NEJMe1208208]

42 **Steil GM**, Agus MS. Tight glycemic control in the ICU - is the earth flat? *Crit Care* 2014; **18**: 159 [PMID: 25041720 DOI: 10.1186/cc13950]

43 **Wernerman J**, Desaive T, Finfer S, Foubert L, Furnary A, Holzinger U, Hovorka R, Joseph J, Kosiborod M, Krinsley J, Mesotten D, Nasraway S, Rooyackers O, Schultz MJ, Van Herpe T, Vigersky RA, Preiser JC. Continuous glucose control in the ICU: report of a 2013 round table meeting. *Crit Care* 2014; **18**: 226 [PMID: 25041718 DOI: 10.1186/cc13921]

44 **Egi M**, Bellomo R, Stachowski E, French CJ, Hart G. Variability of blood glucose concentration and short-term mortality in critically ill patients. *Anesthesiology* 2006; **105**: 244-252 [PMID: 16871057]

45 **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]

46 **Hermanides J**, Vriesendorp TM, Bosman RJ, Zandstra DF, Hoekstra JB, Devries JH. Glucose variability is associated with intensive care unit mortality. *Crit Care Med* 2010; **38**: 838-842 [PMID: 20035218 DOI: 10.1097/CCM.0b013e3181cc4be9]

47 **Brunner R**, Adelsmayr G, Herkner H, Madl C, Holzinger U. Glycemic variability and glucose complexity in critically ill patients: a retrospective analysis of continuous glucose monitoring data. *Crit Care* 2012; **16**: R175 [PMID: 23031322 DOI: 10.1186/cc11657]

48 **Lanspa MJ**, Dickerson J, Morris AH, Orme JF, Holmen J, Hirshberg EL. Coefficient of glucose variation is independently associated with mortality in critically ill patients receiving intravenous insulin. *Crit Care* 2014; **18**: R86 [PMID: 24886864 DOI: 10.1186/cc13851]

49 **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]
50 **Krinsley JS**. Glycemic control in the critically ill - 3 domains and diabetic status means one size does not fit all! *Crit Care* 2013; **17**: 131 [PMID: 23594397 DOI: 10.1186/cc12584]
51 **Tarantino G**, Finelli C. What about non-alcoholic fatty liver disease as a new criterion to define metabolic syndrome? *World J Gastroenterol* 2013; **19**: 3375-3384 [PMID: 23801829 DOI: 10.3748/wjg.v19.i22.3375]

P- Reviewer: Beltowski J, Tarantino G **S- Editor**: Song XX
L- Editor: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v6.i9.1092

World J Diabetes 2015 August 10; 6(9): 1092-1096
ISSN 1948-9358 (online)

EDITORIAL

Interpretation of cardiovascular outcome trials in type 2 diabetes needs a multiaxial approach

Odd Erik Johansen

Odd Erik Johansen, Boehringer Ingelheim Norway KS, 1373 Asker, Norway

Odd Erik Johansen, Department of Medical Research, Bærum Hospital, Vestre Viken Hospital Trust, 1309 Rud, Norway

Author contributions: Johansen OE prepared and wrote this editorial.

Conflict-of-interest statement: I am employed by Boehringer Ingelheim Norway KS.

Correspondence to: Odd Erik Johansen, MD, PhD, Medical Director, Boehringer Ingelheim Norway KS, P.O. Box 405, 1373 Asker, Norway. odd_erik.johansen@boehringer-ingelheim.com
Telephone: +47-91817674
Fax: +47-66761330

Received: January 20, 2015
Peer-review started: January 22, 2015
First decision: March 6, 2015
Revised: June 25, 2015
Accepted: July 21, 2015
Article in press: July 23, 2015
Published online: August 10, 2015

Abstract

In cardiovascular (CV) diabetology a "one-size fits-all" approach needs caution as vasculopathy and CV manifestations in patients with type 2 diabetes (T2D) with short disease duration are different as compared to those with longer duration. This is of relevance when interpreting results of CV outcome trials as responses to any intervention aimed to reduce CV risk might be different in patients with established vasculopathy as compared to those without, where also the duration of the intervention may play a role. Additionally, the mode-of-action of the intervention and its assumed time to peak CV risk modulation need to be taken into account: an intervention with possibly immediate effects, like on blood pressure or other direct functional dynamic parameters such as endothelial function or renal hemodynamics, could likely provide a meaningful impact on CV outcomes over a shorter time span than interventions that primarily target pathways that work on atherosclerotic processes, organ-remodelling, or vessel integrity. We are now faced with CV outcome results to interpret from a plethora of outcomes trials in T2D, some of which are testing the CV risk modulation predominantly beyond glucose lowering, *e.g.*, as is the case for several trials testing the newer therapy classes di-peptidyl peptidase-4 inhibitors, glucagon-like protein-1 receptor analogues and sodium glucose co-transporter-2 inhibitors, and this paper reviews the data that support a call for a multiaxial approach to interpret these results.

Key words: Type 2 diabetes; Pharmaceutical; Risk reduction; Outcomes; Cardiovascular

Core tip: Vasculopathy and cardiovascular (CV) manifestations in patients with type 2 diabetes differ dependent on disease duration. This literature review supports that it is necessary to contextualize results of CV outcome trials in diabetes to diabetes duration as well as duration and mode of action of the intervention, which may be of particular relevance for those interventions that primarily target pathways related to atherosclerotic processes, organ-remodelling, or vessel integrity. Several CV outcome trials testing newer therapy classes (*i.e.*, di-peptidyl peptidase-4 inhibitors,

glucagon-like protein-1 receptor analogues and sodium glucose co-transporter-2 inhibitors) are now due to report and a multiaxial approach to interpret these results is needed.

Johansen OE. Interpretation of cardiovascular outcome trials in type 2 diabetes needs a multiaxial approach. *World J Diabetes* 2015; 6(9): 1092-1096 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i9/1092.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i9.1092

INTERPRETATION OF CARDIOVASCULAR OUTCOME TRIALS IN TYPE 2 DIABETES

The human mind is a master in pattern recognitions. A flip-side to this profound ability in predicting cause-and-effects surfaces however in dealing with complex questions where a "one-size fits-all" approach not necessarily longer applies. Cardiovascular (CV) diabetology is one example of a complex system where a "one-size fits-all" approach needs caution. For example, vasculopathy and CV manifestations in patients with type 2 diabetes (T2D) with short disease duration are different as compared to those with longer T2D duration. Further, the response to any intervention aimed to reduce CV risk might be different in patients with established vasculopathy as compared to those without, where also the duration of the intervention may play a role for a successful risk reduction. The last point is however also dependent on the mode-of-action of the intervention, since an intervention with possibly immediate effects, like on blood pressure or other direct functional dynamic parameters such as endothelial function or renal hemodynamics, likely could provide a meaningful impact on outcomes over a shorter time span than interventions that primarily targets pathways that work on atherosclerotic processes, organ-remodelling, or vessel integrity. These are all important considerations that need to be taken into account when we soon will be faced with results to interpret from a plethora of outcomes trials in T2D, some of which are testing the CV risk modulation potential predominantly beyond glucose lowering, *e.g.*, as is the case for the newer therapy classes di-peptidyl peptidase (DPP)-4 inhibitors, glucagon-like protein-1 receptor analogues and sodium glucose co-transporter-2 inhibitors (Figure 1).

THE RELEVANCE OF CONTEXTUALIZING OUTCOME TRIAL RESULTS TO T2D DURATION AND PRESENCE OR ABSENCE OF CV COMPLICATIONS?

T2D is a progressive complex metabolic disease[1] leading to disturbances in several pathways (*e.g.*, hyperglycemia, insulin resistance, inflammation, oxidation, endothelial dysfunction, dysfunctional adiposity) involved in vasculo-biopathology and CV complications[2]. With this in mind, what could possibly explain differing impact on CV risk of an intervention given early in the T2D disease course *vs* late? One element relates to that longer-standing T2D is associated with silent vasculopathy, as illustrated by *e.g.*, approximately 20% of clinically asymptomatic patients with T2D having significant coronary artery disease, either by invasive coronary angiography[3] or by photon emission-computed tomography myocardial perfusion imaging[4]. Further, since longer duration of the disease and advancing age typically lead to an accumulation of subclinical [such as vascular stiffness[5], coronary artery calcifications (CAC)[6], or myocardial dysfunction[7]] or clinical manifestations of CV complications (*i.e.*, myocardial infarction), or microvascular complications (which is an emerging risk factor for CV complications[8]), it might be conceivable that if the patient population being studied has advanced vasculopathy, the likelihood to influence the disease course could be lower. In particular if end-stage complications have manifested, *e.g.*, as observed in patients on dialysis where statins apparently do not reduce CV risk[9], since these patients may be less sensitive to improvement in CV risk factors.

In longer-term outcome trials in T2D, where different strategies to intensively improve glucose control were tested, this point, to a certain degree, was illustrated by different results on outcomes as observed in the United Kingdom Prospective Diabetes Study (UKPDS); a study[10] that recruited newly diagnosed patients with T2D with a low CV disease burden, and the ORIGIN trial[11], which recruited patients with 5-6 years of diabetes duration of whom approximately 60% had prior CV complications (Figure 2). Although both studies achieved meaningful differences in glucose control between treatment arms, only those patients with newly diagnosed T2D without prevalent CV disease in UKPDS, achieved outcome benefits. Whether this was related to the short diabetes duration and low vasculopathy burden at the start of the intervention, a long treatment duration, or mode of action of the different interventions, is not known. The potential differing response to preventive therapies in patients with short *vs* long standing diabetes was also illustrated in a subanalysis of the recent CV outcome trial comparing outcomes of placebo or alogliptin superimposed on standard of care in patients with T2D and acute coronary syndrome (the EXAMINE trial)[12]. Overall the glycemic differences between the treatment arms were small and the primary outcome was neutral, however, patients with shorter diabetes duration (*i.e.*, less than 5 years) had reduced risk [hazard ratio (HR) = 0.74 (95%CI: 0.54, 1.01)] for the composite primary CV endpoint as compared to those with longer disease duration [5-10 years HR = 0.81 (95%CI: 0.58, 1.13); > 10 years HR = 1.22 (95%CI: 0.98, 1.53); interaction with treatment *P*-value 0.014]. Another interesting observation in the context

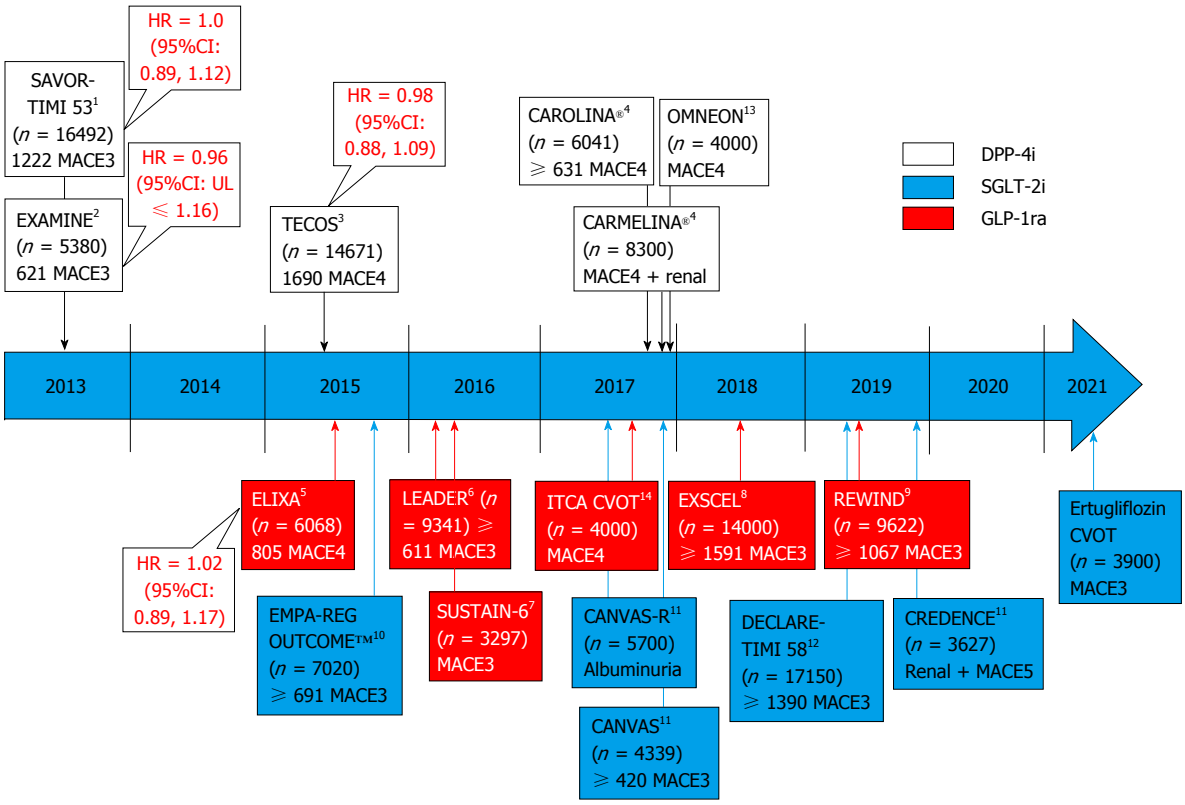

Figure 1 Anticipated ending of outcome trials in type 2 diabetes and their primary outcomes and patient/event numbers involving di-peptidyl peptidase-4 inhibitors, glucagon-like protein-1 receptor analogues and sodium glucose co-transporter-2 inhibitors. Superscript note indicate study drug(s) in testing. All trials are placebo controlled except CAROLINA® that compared *vs* the sulfonylurea glimepiride. [1]Saxagliptin, Astra Zeneca; [2]Alogliptin, Takeda; [3]Sitagliptin, Merck; [4]Linagliptin, Boehringer Ingelheim/Eli Lilly; [5]Lixisenatide, Sanofi Aventis; [6]Liraglutide, Novo Nordisk; [7]Semaglutide, Novo Nordisk; [8]Exenatide, Astra Zeneca; [9]Dulaglutide, Eli Lilly; [10]Empagliflozin, Boehringer Ingelheim/Eli Lilly; [11]Canagliflozin, J and J; [12]Dapagliflozin, Astra Zeneca; [13]Omarigliptin (once weekly tablet), Merck; [14]ITCA 650 [once/twice yearly exenatide *via* subcutaneous mini-pump (Duros device)], Intarcia Therapeutics. DPP-4: Di-peptidyl peptidase-4; GLP-1: Glucagon-like protein-1; SGLT-2: Sodium glucose co-transporter-2; MACE3: Composite endpoint of cardiovascular death, non-fatal myocardial infarction and non-fatal stroke; MACE4: MACE3 plus hospitalized unstable angina pectoris; MACE5: MACE4 plus hospitalized congestive heart failure.

of degree of vasculopathy as a potential determinant for the effect of an intervention stems from the veterans affairs diabetes trial (VADT)[13]. The VADT tested whether intensive glucose control (targeted/achieved HbA1c < 6.0%/6.9%) *vs* conventional (targeted/achieved HbA1c < 9.0%/8.9%) could reduce CV risk in 1791 patients with long-standing T2D[13]. Although intensive glucose-lowering therapy did not significantly reduce CV events in the study cohort as a whole, there was evidence that the response was modified by baseline CAC. They observed, *e.g.*, that among those randomized to intensive treatment, in the subgroup with CAC > 100, 11 of 62 individuals had events, while only 1 of 52 individuals with CAC ⩽ 100 had an event (significant risk reduction), indicating that intensive glucose lowering reduced CV events only in those with less extensive calcified coronary atherosclerosis[14].

WHY IS IT IMPORTANT TO CONTEXTUALIZE OUTCOME TRIAL RESULTS TO DURATION OF INTERVENTION?

In order for an intervention to reduce CV risk it has to interfere with the cascade of events that lead to complications. Since T2D is a CV risk entity by itself, where CV risk typically is further magnified in the presence of CV complications, any intervention that targets outcomes like myocardial infarction or hospitalization for angina pectoris primary related to atherosclerosis, likely have to be of sufficient duration since the biopathological processes typically might evolve over decades[15,16]. Although the targeted study outcome as well as the mode of action of the intervention certainly plays an important role here, one important question is when the effects of an intervention are assumed to peak. This was illustrated, for example, by the PRO active trial[17], comparing pioglitazone *vs* placebo as secondary CV prevention: at study end the primary endpoint just missed the significance level, but as the survival curves separated in favour of pioglitazone towards study end, it was speculated that the trial result could have looked different if the trial had run longer[18]. At this point it is only speculations if the two other recent neutral outcome trials involving DPP-4 is, a class that in animal studies have been implied to reduce several pathways leading to atherosclerosis[19], namely SAVOR-TIMI53[20], EXAMINE[12], and TECOS[21] would have showed different results if ran longer than

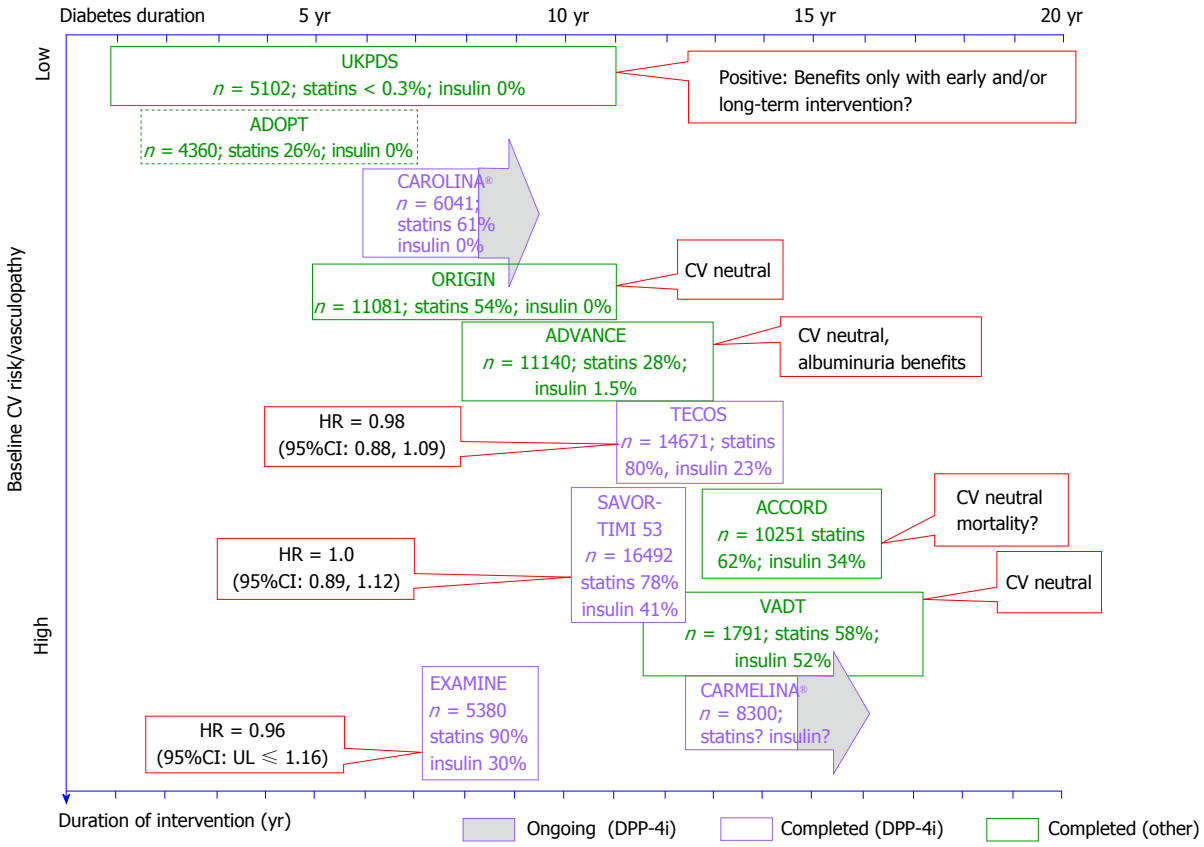

Figure 2 Selected outcome trials in type 2 diabetes with a focus on di-peptidyl peptidase-4 inhibitor studies, and their results, in the context of the duration of intervention and the study population's diabetes duration and baseline cardiovascular risk. CV: Cardiovascular; UKPDS: United Kingdom Prospective Diabetes Study; VADT: Veterans affairs diabetes trial.

their median duration of respectively 2.1, 1.5 and 2.8 years. Obviously this needs further clarification in trials of longer duration.

RESULTS OF CV OUTCOME TRIALS IN T2D NEED TO BE INTERPRETED IN A MULTIDIMENSIONAL FRAME

Over the next years, with several CV outcome trials due to report (Figure 1)[22-29], an opportunity for great learnings is at our doorsteps. Since some trials might even contribute to paradigm shifts in our approach to T2D management, it is important to contextualize the results to the study populations in scope taking into account T2D disease duration, degree of vasculopathy, duration of the intervention, and the mode of action of the intervention (Figure 2). Only this will fully support and facilitate an optimized patient centered approach to T2D care and CV risk management[30].

REFERENCES

1 **Defronzo RA**. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* 2009; **58**: 773-795 [PMID: 19336687 DOI: 10.2337/db09-9028]

2 **Johansen OE**. Cardiovascular disease and type 2 diabetes mellitus: a multifaceted symbiosis. *Scand J Clin Lab Invest* 2007; **67**: 786-800 [PMID: 17852797 DOI: 10.1080/00365510701408558]

3 **Johansen OE**, Birkeland KI, Orvik E, Flesland Ø, Wergeland R, Ueland T, Smith C, Endresen K, Aukrust P, Gullestad L. Inflammation and coronary angiography in asymptomatic type 2 diabetic subjects. *Scand J Clin Lab Invest* 2007; **67**: 306-316 [PMID: 17454845 DOI: 10.1080/00365510601045088]

4 **Wackers FJ**, Young LH, Inzucchi SE, Chyun DA, Davey JA, Barrett EJ, Taillefer R, Wittlin SD, Heller GV, Filipchuk N, Engel S, Ratner RE, Iskandrian AE. Detection of silent myocardial ischemia in asymptomatic diabetic subjects: the DIAD study. *Diabetes Care* 2004; **27**: 1954-1961 [PMID: 15277423 DOI: 10.2337/diacare.27.8.1954]

5 **Prenner SB**, Chirinos JA. Arterial stiffness in diabetes mellitus. *Atherosclerosis* 2015; **238**: 370-379 [PMID: 25558032 DOI: 10.1016/j.atherosclerosis.2014.12.023]

6 **Cox AJ**, Hsu FC, Freedman BI, Herrington DM, Criqui MH, Carr JJ, Bowden DW. Contributors to mortality in high-risk diabetic patients in the Diabetes Heart Study. *Diabetes Care* 2014; **37**: 2798-2803 [PMID: 24989706 DOI: 10.2337/dc14-0081]

7 **Ofstad AP**, Urheim S, Dalen H, Orvik E, Birkeland KI, Gullestad L, W Fagerland M, Johansen OE, Aakhus S. Identification of a definite diabetic cardiomyopathy in type 2 diabetes by comprehensive echocardiographic evaluation: A cross-sectional comparison with non-diabetic weight-matched controls. *J Diabetes* 2014; Epub ahead of print [PMID: 25350248 DOI: 10.1111/1753-0407.12239]

8 **Juutilainen A**, Lehto S, Rönnemaa T, Pyörälä K, Laakso M. Retinopathy predicts cardiovascular mortality in type 2 diabetic men and women. *Diabetes Care* 2007; **30**: 292-299 [PMID: 17259497 DOI: 10.2337/dc06-1747]

9 **Palmer SC**, Navaneethan SD, Craig JC, Johnson DW, Perkovic V, Nigwekar SU, Hegbrant J, Strippoli GF. HMG CoA reductase inhibitors (statins) for dialysis patients. *Cochrane Database Syst Rev* 2013; **9**: CD004289 [PMID: 24022428 DOI: 10.1002/14651858.

CD004289.pub5]
10 **Holman RR**, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med* 2008; **359**: 1577-1589 [PMID: 18784090 DOI: 10.1056/NEJMoa0806470]
11 **Gerstein HC**, Bosch J, Dagenais GR, Díaz R, Jung H, Maggioni AP, Pogue J, Probstfield J, Ramachandran A, Riddle MC, Rydén LE, Yusuf S. Basal insulin and cardiovascular and other outcomes in dysglycemia. *N Engl J Med* 2012; **367**: 319-328 [PMID: 22686416 DOI: 10.1056/NEJMoa1203858]
12 **White WB**, Cannon CP, Heller SR, Nissen SE, Bergenstal RM, Bakris GL, Perez AT, Fleck PR, Mehta CR, Kupfer S, Wilson C, Cushman WC, Zannad F. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N Engl J Med* 2013; **369**: 1327-1335 [PMID: 23992602 DOI: 10.1056/NEJMoa1305889]
13 **Reaven PD**, Moritz TE, Schwenke DC, Anderson RJ, Criqui M, Detrano R, Emanuele N, Kayshap M, Marks J, Mudaliar S, Harsha Rao R, Shah JH, Goldman S, Reda DJ, McCarren M, Abraira C, Duckworth W. Intensive glucose-lowering therapy reduces cardiovascular disease events in veterans affairs diabetes trial participants with lower calcified coronary atherosclerosis. *Diabetes* 2009; **58**: 2642-2648 [PMID: 19651816 DOI: 10.2337/db09-0618]
14 **Duckworth W**, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, Zieve FJ, Marks J, Davis SN, Hayward R, Warren SR, Goldman S, McCarren M, Vitek ME, Henderson WG, Huang GD. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med* 2009; **360**: 129-139 [PMID: 19092145 DOI: 10.1056/NEJMoa0808431]
15 **Dzau V**, Braunwald E. Resolved and unresolved issues in the prevention and treatment of coronary artery disease: a workshop consensus statement. *Am Heart J* 1991; **121**: 1244-1263 [PMID: 2008853 DOI: 10.1016/0002-8703(91)90694-D]
16 **Lee SJ**, Leipzig RM, Walter LC. Incorporating lag time to benefit into prevention decisions for older adults. *JAMA* 2013; **310**: 2609-2610 [PMID: 24322396 DOI: 10.1001/jama.2013.282612]
17 **Dormandy JA**, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, Skene AM, Tan MH, Lefèbvre PJ, Murray GD, Standl E, Wilcox RG, Wilhelmsen L, Betteridge J, Birkeland K, Golay A, Heine RJ, Korányi L, Laakso M, Mokán M, Norkus A, Pirags V, Podar T, Scheen A, Scherbaum W, Schernthaner G, Schmitz O, Skrha J, Smith U, Taton J. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* 2005; **366**: 1279-1289 [PMID: 16214598 DOI: 10.1016/S0140-6736(05)67528-9]
18 **Erdmann E**, Dormandy J, Wilcox R, Massi-Benedetti M, Charbonnel B. PROactive 07: pioglitazone in the treatment of type 2 diabetes: results of the PROactive study. *Vasc Health Risk Manag* 2007; **3**: 355-370 [PMID: 17969365]
19 **Fadini GP**, Avogaro A. Cardiovascular effects of DPP-4 inhibition: beyond GLP-1. *Vascul Pharmacol* 2011; **55**: 10-16 [PMID: 21664294 DOI: 10.1016/j.vph.2011.05.001]
20 **Scirica BM**, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, Ohman P, Frederich R, Wiviott SD, Hoffman EB, Cavender MA, Udell JA, Desai NR, Mosenzon O, McGuire DK, Ray KK, Leiter LA, Raz I. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med* 2013; **369**: 1317-1326 [PMID: 23992601 DOI: 10.1056/NEJMoa1307684]
21 **Green JB**, Bethel MA, Armstrong PW, Buse JB, Engel SS, Garg J, Josse R, Kaufman KD, Koglin J, Korn S, Lachin JM, McGuire DK, Pencina MJ, Standl E, Stein PP, Suryawanshi S, Van de Werf F, Peterson ED, Holman RR. Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med* 2015; **373**: 232-242 [PMID: 26052984 DOI: 10.1056/NEJMoa1501352]
22 **Inzucchi SE**, Zinman B, Wanner C, Ferrari R, Fitchett D, Hantel S, Espadero RM, Woerle HJ, Broedl UC, Johansen OE. SGLT-2 inhibitors and cardiovascular risk: proposed pathways and review of ongoing outcome trials. *Diab Vasc Dis Res* 2015; **12**: 90-100 [PMID: 25589482 DOI: 10.1177/1479164114559852]
23 **Marx N**, Rosenstock J, Kahn SE, Zinman B, Kastelein JJ, Lachin JM, Espeland MA, Bluhmki E, Mattheus M, Ryckaert B, Patel S, Johansen OE, Woerle HJ. Design and baseline characteristics of the CARdiovascular Outcome Trial of LINAgliptin Versus Glimepiride in Type 2 Diabetes (CAROLINA®). *Diab Vasc Dis Res* 2015; **12**: 164-174 [PMID: 25780262 DOI: 10.1177/1479164115570301]
24 Cardiovascular and Renal Microvascular Outcome Study With Linagliptin in Patients With Type 2 Diabetes Mellitus (CARMELINA). [Accessed 2015 Mar 21]. Available from: URL: https://www.clinicaltrials.gov/ct2/show/NCT01897532?term=carmelina&rank=1
25 **Marso SP**, Poulter NR, Nissen SE, Nauck MA, Zinman B, Daniels GH, Pocock S, Steinberg WM, Bergenstal RM, Mann JF, Ravn LS, Frandsen KB, Moses AC, Buse JB. Design of the liraglutide effect and action in diabetes: evaluation of cardiovascular outcome results (LEADER) trial. *Am Heart J* 2013; **166**: 823-30.e5 [PMID: 24176437 DOI: 10.1016/j.ahj.2013.07.012]
26 Result presentation of evaluation of LIXisenatide in acute coronary syndrome, a long-term cardiovascular end point trial of lixisenatide versus placebo. American Diabetes Association annual meeting. United States: Boston, 2015
27 Exenatide study of cardiovascular event lowering trial (EXSCEL): A trial to evaluate cardiovascular outcomes after treatment with exenatide onceweekly in patients with type 2 diabetes mellitus. [Accessed 2015 Mar 21]. Available from: URL: https://www.clinicaltrials.gov/ct2/show/NCT01144338?term=exenatide cardiovascular&rank=1
28 Researching Cardiovascular Events With a Weekly Incretin in Diabetes (REWIND). [Accessed 2015 Mar 21]. Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01394952?term=NCT01394952&rank=1
29 A study to evaluate cardiovascular outcomes in patients with type 2 diabetes treated with ITCA 650. [Accessed 2015 Mar 21]. Available from: URL: https://www.clinicaltrials.gov/ct2/show/NCT01455896?term=itca&rank=3
30 **Inzucchi SE**, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2015; **38**: 140-149 [PMID: 25538310 DOI: 10.2337/dc14-2441]

P- Reviewer: Gómez-Sáez J, Pirola L **S- Editor**: Gong XM
L- Editor: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v6.i9.1097

World J Diabetes 2015 August 10; 6(9): 1097-1107
ISSN 1948-9358 (online)

REVIEW

Aloesin as a medical food ingredient for systemic oxidative stress of diabetes

Mesfin Yimam, Lidia Brownell, Qi Jia

Mesfin Yimam, Lidia Brownell, Qi Jia, Unigen, Inc., Seattle, WA 98121, United States

Author contributions: All authors contributed to this manuscript.

Conflict-of-interest statement: Mesfin Yimam, Lidia Brownell and Qi Jia are currently Unigen employees and therefore, have competing financial interests. In addition, Qi Jia is the co-inventor of US issued patent entitled 7-Hydroxy Chromones as Potent Anti-oxidant.

Correspondence to: Mesfin Yimam, DVM, MS, Unigen, Inc., 3005 1st Ave, Seattle, WA 98121, United States. myimam@unigen.net
Telephone: +1-360-4868200
Fax: +1-360-4139135

Received: April 22, 2015
Peer-review started: April 22, 2015
First decision: May 13, 2015
Revised: July 1, 2015
Accepted: July 24, 2015
Article in press: July 27, 2015
Published online: August 10, 2015

Abstract

Diabetes is a chronic disease that requires a long term management where oxidative stress plays a pivotal role in disease progression and intensifying secondary complications. In spite of all the research on diabetes and recent advances in diabetes treatments, the reality is that there is no cure for diabetes and its devastating complications. While currently available anti-diabetic therapies are effective in reducing blood glucose level, they are not without associated side effects when they are used for a long term applications. As a result, physicians and patients are inclining more towards to a safer therapy with less serious side effects in the form of medicinal foods and botanical alternatives that are suitable for chronic usage. Aloesin, an Aloe chromone, has previously been formulated with an aloe polysaccharide to give a composition called Loesyn, where it showed significant impact in reducing glycosylated hemoglobin, fasting blood glucose, fructosamine and plasma insulin level in humans. Radical scavenging activities of chromones and polysaccharides from Aloe have also been reported. Here we rationalize the relevance of use of Aloesin alone or in a standardized blend with Aloe polysaccharides, as a potential medical food to manage systemic oxidative stress and/or high blood glucose of diabetes.

Key words: Aloesin; Aloe polysaccharides; Diabetes; Oxidative stress; Medicinal food; Aloe chromone

Core tip: Diabetes has become epidemic in industrialized countries; Diabetes is a chronic disease with no cure; Oxidative stress plays a pivotal role in diabetes complication; Aloesin and aloe polysaccharides have strong free radical scavenging activities; Aloesin formulated with aloe polysaccharides has shown merits in diabetes management in human clinical trials; Aloesin formulated with aloe polysaccharides could have potentials in combating diabetes associated oxidative stress or to be used as an adjunct to pharmaceutical drugs.

Yimam M, Brownell L, Jia Q. Aloesin as a medical food ingredient for systemic oxidative stress of diabetes. *World J Diabetes* 2015; 6(9): 1097-1107 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i9/1097.htm DOI: http://

dx.doi.org/10.4239/wjd.v6.i9.1097

INTRODUCTION

The diabetes epidemic that continues to sweep across the United States has left an estimated 29.1 million Americans in 2012 struggling with the disease. Currently, 387 million people worldwide are affected by diabetes mellitus and are predicted to reach 592 million in 2035. A staggering number, 4.9 million deaths were directly caused by diabetes in the year 2014 highlighting the death of a human being every seven seconds due to the seriousness of the disease. It is also predicted to be the 7th leading cause of death by the year 2030[1]. There are 86 million people in the United States who have elevated blood glucose levels and worldwide, more than 300 million people were estimated to have this pre-diabetic condition[2]. Based on the survey carried out in the years between 2009-2012, and fasting glucose or glycosylated hemoglobin (HbA1C) levels, 37% of United States adults age ⩾ 20 years had pre-diabetes. Correcting this percentage to the entire United States population, in 2012 there were an estimated 86 million Americans age 20 years or older with pre-diabetes. Worldwide, by 2025, the pre-diabetic population number is expected to reach over 500 million people, but even more alarming is the fact that between 29%-68% of people with pre-diabetes develops type Ⅱ diabetes over the course of 3-5 years[3]. Diabetes can affect many parts of the body where oxidative stress induced by hyperglycemia is involved in both the development and progression of the disease and can lead to serious complications such as blindness, kidney damage, lower-limb amputations, and cardiovascular diseases.

According to the National Diabetes Statistics Report, 2014, diabetes in the fiscal year of 2012 cost the United States $245 billion as a result of direct medical care (176 billion) and indirect costs (69 billion) due to disability, work loss, and premature death which accounts for more than 10% of all United States health care spending by the government and public. This is a 41% increase from previous estimate of $174 billion in 2007. In 2012, it was estimated that after adjusting for population age and sex differences, average medical expenditures among people with diagnosed diabetes were 2.3 times higher than people without diabetes[4].

Diabetes is one of the largest therapeutic segments of global pharmaceutical sales. It has been projected that the overall annual global spending on medicines will reach nearly $1.2 trillion by 2016 where the top 20 therapy areas will account for 42% of global spending, led by cancer, diabetes and asthma/Chronic Obstructive Pulmonary Disease from which spending on conventional medicines for diabetes expected to range $48-53 billion[5]. Overall, anti-diabetic drugs sales are projected to grow dramatically over the coming years as the addressable patient population continues to increase and new premium priced products enter the market to address high unmet clinical needs. While Food and Drug Administration approved effective drug therapies are currently available, their chronic usages are limited by serious side effects for managing long-term condition of the disease. Hence, both physicians and patients are increasingly seeking safer therapy with less serious side effects in the form of medicinal foods and botanical drugs that are suitable for long term chronic usage to help manage their blood sugar levels. Such safer alternatives would also be appropriate interventions at the pre-diabetic condition to halt or slow progression to full blown type 2 diabetes. Here we describe the relevance of use of an Aloe chromon, Aloesin by itself or in a standardized blend with Aloe polysaccharides as potential medical food ingredients to manage systemic oxidative stress of diabetes and/or mitigating the primary causes as a partial fulfilment to the unmet needs of botanical interventions.

SYSTEMIC OXIDATIVE STRESS IS ASSOCIATED WITH DIABETES AND ITS COMPLICATIONS

Principally, it is recognized that oxidative stress is an imbalance between the production of free radicals and the inherent capacity of the body to counteract or neutralize their harmful effects through interaction with various reducing and sequestering endogenous antioxidant defense networks. Reactive oxygen species (ROS) are heterogeneous population of molecules that include oxygen related free radicals, and non-radical species. Normally, ROS can be generated as by-products of glucose or free fatty acid metabolic processes in the mitochondria. In mitochondrial respiration process, between 0.4%-4% of all oxygen consumed in metabolism of glucose is converted into the free radical superoxide ($O_2^{\cdot}$). Additionally, ROS can also be generated from food additives, environmental sources, (*e.g.*, ultraviolet radiation) and tobacco smoke, and many other environment pollutants. When there is a lack of an appropriate adaptation by the body antioxidant defense system, ROS buildup will lead to the activation of stress-sensitive intracellular signaling pathways that, in turn, promote cellular damage and contribute to the diabetic complications development and progression.

Currently there are considerable indications that multiple biochemical pathways are activated by hyperglycemia, and are associated with the generation of ROS, which ultimately lead to increased oxidative stress. Primarily, chronic elevation of glucose in association with free fatty acid (FFA) can cause oxidative stress due to increased production of mitochondrial ROS, non-enzymatic glycation of proteins, glucose oxidation, increased mitochondrial uncoupling and beta-oxidation. The oxidative stress from both metabolism of glucose and FFA can activate signaling pathways such as nuclear factor-κB (NF-κB), p38 mitogen-activated

protein kinase (MAPK) and NH2-terminal c-Jun kinases. These stress activated pathways, in turn, can lead to insulin resistance, beta-cell dysfunction and impaired insulin secretion proceeding to further damage of the eye, kidney, nerve, cardiovascular system and other complications of type-2 diabetes[6]. This fact holds true even for type-1 diabetes, where systemic oxidative stress is also present[7]. For example, under a clinical study, patients with diabetes mellitus showed a positive correlation of NF-κB activation in peripheral blood mononuclear cells with poor glycemic control.

Under normal circumstances, cells have specific mechanisms to preserve homeostasis[8] that include the synthesis and recycling of γ-glutamyl-cysteinyl-glycine (Glutathion GSH) and enzymes, such as superoxide dismutase (SOD), GSH peroxidase and catalase[9]. However, changes in diet, lifestyle, and aging could result in imbalance between the generation and clearance of ROS. Such excess formation and insufficient removal of the mitochondrial ROS expose the intracellular environment to subsequent oxidative stress challenge.

One of the intracellular mechanisms in response to oxidative stress is the activation of the transcriptional factors, such as NF-κB and activator protein 1, which contribute to changes in many gene responses[10] and play very important roles in mediating immune and inflammatory responses and apoptosis[11]. NF-κB regulates the expression of large number of genes, including pro-inflammatory cytokines, vascular endothelial growth factor, and multiple serine kinase cascades, such as p38 MAPKs which play a significant role in diabetes progression and complications. For instance, insulin receptor (IR) and the IR substrate (IRS) family of proteins are potential targets for the elevated serine kinase. Their involvement was demonstrated in muscle cell model, where activation of p38 MAPK by oxidative stress was found to be linked to the ROS-mediated inhibition of insulin-stimulated glucose transport[12]. In fact, inhibition of insulin signaling was reversed by a specific inhibitor of p38 MAPK.

Oxidative stress in diabetes mellitus causes several adverse effects on the cellular physiology where it is particularly relevant and critical for those tissues that have lower levels of intrinsic antioxidant defenses such as islets. Pertaining to blood glucose level signaling and insulin secretion mediations, β-cells are particularly susceptible to the damages inflicted by oxidative stress due to the fact that ROS cascade eventually will cause induced auto-immune attack, which further accelerate the dysfunction and destruction of β-cells[13] that lead both insulin resistance and impaired insulin secretion[14].

Diabetic peripheral neuropathy is the most common complication of long-standing diabetes mellitus. Neuropathy frequently results in clinically significant morbidities, such as pain, loss of sensation, foot ulcers, gangrene and amputations[15]. It now seems that the pathogenesis of diabetic neuropathy is heterogeneous with causative factors, including microvascular insufficiency, oxidative stress, nitrosative stress, defective neurotrophism, and autoimmune-mediated nerve destruction. As such, oxidative stress has been viewed as a core and fundamental causing factor in the pathogenesis of diabetic neuropathy. Studies have showed proteins that are damaged by oxidative stress have decreased biological activity leading to loss of energy metabolism, cell signaling, transport, and, ultimately, to cell death[16]. Those oxidative stress induced damages have been demonstrated on cell based[17], *in vivo* animals[18], and human clinical studies[19]. Under clinical observations, the impaired glucose tolerance[20] and advanced glycation end products[21] are positively associated with the development and progress of the oxidative stress and neuropathy. As a result, new therapies are aimed at the underlying pathogenesis as well as the symptom complex[22]. For example, anti-oxidants, such as alpha-lipoic acid[23,24], dietary glutathione[25], and polyphenols from grape seeds[26] have shown beneficial clinical effects in management of peripheral nerves function in diabetic rats and human subjects.

One of the common microvascular complications of diabetes, diabetes retinopathy is classified as proliferative and nonproliferative diabetic retinopathy, mainly characterized by retinal neovascularization leading to blindness. It has been estimated that, ones diagnosed, nearly all patients with type 1 diabetes and more than 60% of patients with type 2 diabetes are expected to experience some form of retinopathy by the their first decade[27]. The pathophysiology of diabetic retinopathy has been thought to incorporate multiple intertwined biochemical pathways as key contributors in the development of the disease. Among these, an oxidative stress induced by hyperglycemia has been identified as one of the key players in both the development and progression of the disease[28]. Research has shown that in diabetes patients, besides the increased generation of mitochondrial reactive species (oxygen and nitrogen), the level of antioxidant defence enzymes responsible for scavenging free radicals and maintaining redox homeostasis such as SOD, glutathione reductase, glutathione peroxidase, and catalase were found reduced in the retina[29].

Recently Fiorentino *et al*[30] have summarized the association of diabetes induced ROS as a risk factors for the development of cardiovascular disease. In this review, hyperglycemia was identified as the core of the primary disease and its secondary complications. They propose multiple mechanisms *via* activation of protein kinase C, polyol and hexosamine pathways, and advanced glycation end products production. These pathways, together with hyperglycemia-induced mitochondrial dysfunction and endoplasmic reticulum stress, causes ROS buildup which, in turn, cause cellular damage and contribute to the diabetic complications development and progression[30].

Currently, diabetic nephropathy is largely considered as the leading cause of end-stage renal disease in the western world. Hyperglycemia-mediated alterations of intracellular metabolism, including oxidative stress

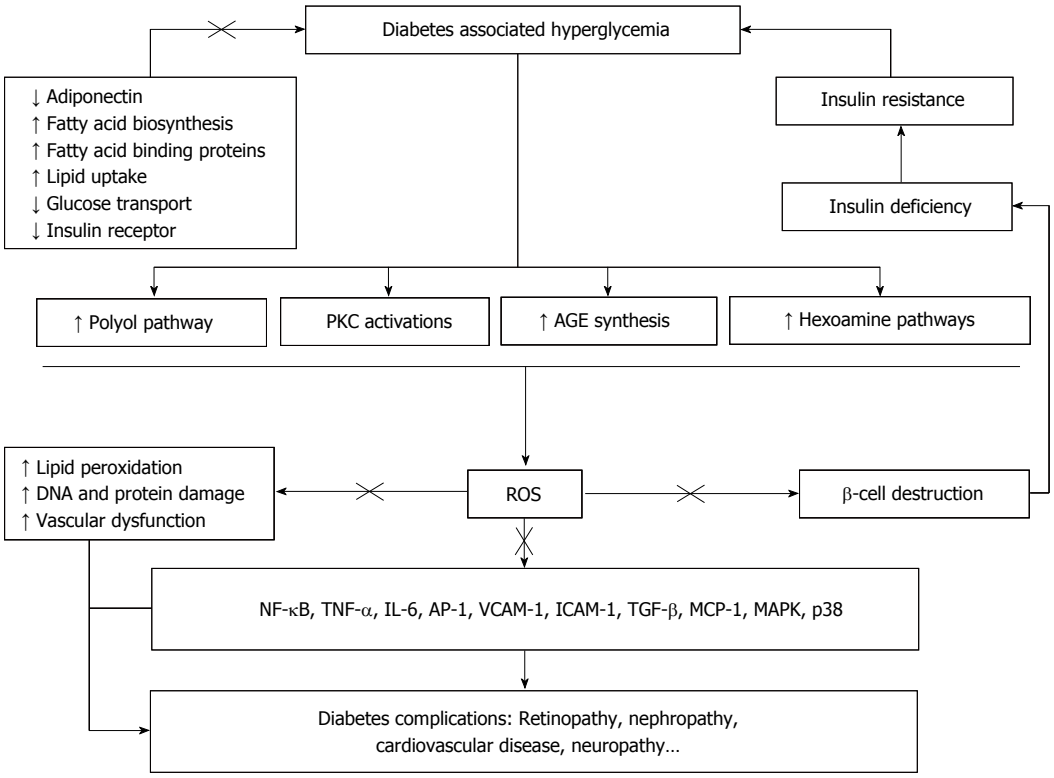

Figure 1 Oxidative stress and its possible pathways leading to diabetes complications. "×" potential sites where aloesin may likely interfere. PKC: Protein kinase C; AGE: Advanced glycation end-products; ROS: Reactive oxygen species; NF-κB: Nuclear factor-kappaB; TNF-α: Tumor necrosis factor alpha; IL-6: Interleukin 6; AP-1: Activating protein-1; VCAM-1: Vascular cell adhesion molecule-1; ICAM-1: Intercellular adhesion molecule-1; TGF-β: Transforming growth factor beta; MCP-1: Monocyte chemotactic protein-1; MAPK, p38: Mitogen-activated protein kinases, p38.

are major contributing factors to the pathogenesis of diabetic nephropathy. Despite the fact that interventions such as intensive lifestyle modification coupled with aggressive therapeutic management of glycemic control, blood pressure control, and inhibition of the renin-angiotensin-aldosterone system have shown promise to slow down progression of the disease, the number of patients with diabetes that ultimately develop end-stage renal disease have become significantly high. These highly predictive consequences suggest that there still is an urgent need to further understand the pathogenesis of the disease in order to establish new therapeutic strategies and promote enhanced clinical management for a better prognosis. In this respect, in the past few years, significant evidences from pre-clinical and clinical studies have been documented to link impaired autophagic activity in the pathogenesis of diabetic renal disease[31]. Autophagy is a fundamental homeostatic cellular process that plays a critical role in maintaining functional integrity during normal or diseased state[32]. It is believed that increase in ROS can induce autophagy, presumably as an adaptive response to cellular stress, and in turn autophagy could lead to reduction of ROS to protect the kidney under diabetic conditions. In fact a recent study has shown this association in a way that exposure of podocytes to a high glucose challenge resulted in an increase in ROS generation and hence autophagy inductions within 24 h. Interestingly, treatment with antioxidant acetylcysteine inhibited the high glucose-induced autophagy[33].

Overall, it has been considered that oxidative stress as a "unifying mechanism" which connects almost all of the complicated destructive biochemical pathways induced by hyperglycemia in diabetic patients[34]. The hypothesis details that besides inducing NF-κB dependent pro-inflammatory and pro-coagulant pathways, mitochondrial-derived ROS to cause breaks in DNA strand which in turn activates poly-(ADP-ribose)-polymerase (PARP). The activation of PARP inhibits glyceraldehyde phosphate dehydrogenase activity which causes the accumulation of glycolytic intermediates. The intermediates then flux into the advanced glycation endproducts, protein kinase C, polyol, and hexosamine pathways, in part, are the major biochemical pathways of diabetes complications development and progression. The possible pathways have been summarized in Figure 1.

With the strong scientific and clinical evidence to link the impaired insulin sensitivity, beta-cell dysfunction, and diabetes complications directly with oxidative stress, new therapeutic approaches by administration of anti-oxidants[35] or modulation of the oxidative-inflammatory cascade[36] have been proposed. It is likely a promising approach to incorporate systemic oxidative stress management into clinical practice in order to control the contributing factor of diabetes and its complications[37].

DISTINCTIVE NUTRITIONAL REQUIREMENTS TO MANAGE THE OXIDATIVE STRESS

Anti-oxidant defense systems are species specific and are prone to changes in nutrition; for example ascorbic acid and α-tocopherol cannot be synthesized by humans and therefore, needs to be acquired from consumed diet[38]. Vitamins, minerals, amino acids, phenolic acids, flavanoids, anthrocynadines, pycnogenol, coumarine derivatives, polyphenols and many different types of herbal extracts[39] have been promoted as types of antioxidant products. In functional specificity: (1) Dietary antioxidants: The beneficial effects of dietary antioxidants, such as resveratrols[40] and alpha-lipoic acid[41] in reducing the incidence of coronary heart diseases; butylated hydroxytoluene and β-carotene in photocarcinogenesis[42] have been documented. Nevertheless, while antioxidants may reduce free radicals generated by radiotherapy and chemotherapy, clinical evidences are limited to show their significant applications in reducing systemic oxidative stress, even at higher dosages[43,44]; (2) Vitamins and Minerals: Common antioxidants, such as vitamins A, C, E, mixed carotenoids, Co-Q10, α-lipoic acid, bio-flavonoids, antioxidant minerals (copper, zinc, manganese and selenium) and other cofactors (folic acid, vitamins B1, B2, B6, and B12) have been evaluated in streptozotocin and alloxan induced diabetes models[45]. Increased glutathione, catalase and SOD activities, reduced lipid peroxidation, and reduced oxidative stress markers functions on experimentally induced diabetic animal models have been reported[46]. Despite the significant findings from animal diabetes models, clinical trials conducted to date failed to provide adequate support for the use of antioxidants such as vitamin E, vitamin C, beta-carotene, selenium in a period of 7.5-12.5 years to reduce the risks of diabetes and to prevent its complications in randomized placebo-controlled clinical trials[47]. The failure to deliver the perceived reduction of systemic oxidative stress from supplement of simple anti-oxidant vitamins may be due to the sub-optimum dosages, poor bioavailability, and lacks of organ/tissue specificity from the antioxidants. Another factor that has to be taken into consideration is how to better control the macronutrients that induce oxidative stress[48]. In fact, a study conducted using foods selected based on total antioxidant capacity without standardization was failed to achieve the reductions of oxidative stress markers in a crossover two weeks intervention study[49]; and (3) Polyphenols: Polyphenols are classes of natural anti-oxidants that exist in fruits, vegetables, nuts, different plant part as free radical scavengers, that prevent free radical chain reactions by counteracting existing free radicals and/or upholding a reducing environment around the cells[50]. To deliver natural polyphenols in medical foods and in order to meet distinctive nutritional requirements, managing the oxidative stress has unique advantage than administration of classical anti-oxidation vitamins. Natural polyphenols have a great structural diversity with anti-oxidation capacities higher than vitamin C and E[51]. The food sources, daily intakes and related bioavailability of polyphenols have been very well documented[52]. The polyphenols in foods can be quantitatively analyzed using modern analytical tools with the complement test of free radical scavenging activity using diphenylpicrylhydrazyl (DPPH) assay[53]. However, both the complicated polyphenol compositions in food matrix[54] and changes of the chemically active polyphenols into polymerized or decomposed compounds in food processing and storing make the delivery of standardized polyphenols with consistency a very challenging task[55]. Those challenges may give explanations for the observations in two prospective human clinical studies that showed daily intake from 8.85 to 47.2 mg total flavonoids from flavonoid-rich foods, such as apple, tea, berries, citrus, broccoli, red wines, were not associated with the risk of type 2 diabetes[56]. Quercetin, a high polarity but low bio-available flavonoid glycoside, was the major contributor to the total flavonoids (72%) in the foods.

On the other hand, in another clinical trial, 30% lower risk of developing type-2 diabetes from women who ate more than 1 apple per day or had more than 4 cups of tea than those who consumed no apple or tea were observed which shined a promising light[57] for the potential use of antioxidants in diabetes prevention. This leads the possibility of selecting specific types of polyphenols with an improved bioavailability, potent anti-oxidation properties and standardized dosage level to deliver the perceived health benefits to diabetic patients by managing systemic oxidative stress.

SCIENTIFIC EVIDENCE TO SUPPORT THE POTENTIAL USAGE OF ALOESIN AND ALOE POLYSACCHARIDES AS A MEDICAL FOOD INGREDIENTS TO MEET THE DISTINCTIVE NUTRITIONAL REQUIREMENTS OF DIABETES

Aloe plants and extracts have been utilized for diabetes

Aloe vera (*Aloe barbadensis* Miller) is a perennial cactus like succulent plant belonging to the Xanthorrhoeaceae family. It is a biochemically complex plant that includes more than 300 species comprising many biologically active substances with diverse applications[58]. The major components of *Aloe vera* such as chromones, anthraquinones, polysaccharides, vitamins, enzymes, and low molecular weight substances, such as organic acids and minerals, collectively, have been reported to possess immunomodulatory, anti-inflammatory, ultraviolet radiation protective, antiprotozoal, and wound/burn-healing promoting properties[59]. While polysaccharides, in specific, have been described to show anti-inflammation, anticancer, and immunomodulation

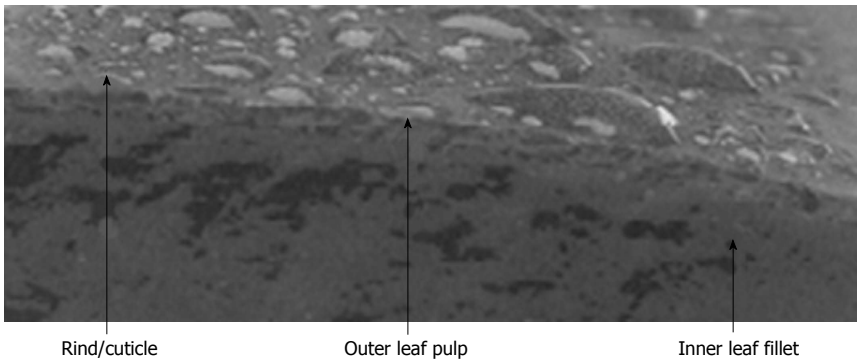

Figure 2 Cross-section of Aloe.

activities, biological activities such as cell growth stimulation, melanin synthesis inhibitions and antioxidant functions were documented for aloesin[60]. Structurally, the aloe whole leaf encompasses three main distinctive sections each with specific function. These parts are categorized as the green rind or cuticle, the outer leaf pulp and the gel fillet (Figure 2). Polysaccharides are mainly located within the mucilaginous gel from the parenchymatous tissue whereas aloesin is housed inside the exudate of the leaf pulp.

Significant animal studies have reported beneficial effects of *Aloe vera* including reduced fasting blood glucose levels in alloxan-induced diabetic mice[61]; improved glucose tolerance in glucose-loaded rats[62]; decreased glucose levels[63] and, enhanced liver gluconeogenesis in streptozotocin-induced diabetic rats[64]; decreased oxidative damage in the brains of streptozotocin-induced diabetic mice[65]; decreased lipid peroxidation in diabetic rat kidney[66] and liver[67]; and, in streptozotocin-induced diabetic rats, decreased fasting glucose, normalization of lipids and liver and kidney fatty acid composition with reduced liver transaminases, and improved plasma insulin levels[63]. Articles on systematic review of herbs and dietary supplements for glycemic control in diabetes and a systematic review of aloe's clinical effectiveness give substantial information regarding use of aloe in diabetes[68,69].

In contrast to animal studies, until recently few human clinical trials were found in the literature. The two studies most frequently cited to support the use of *Aloe* for human diabetes[70,71] contain methodological flaws, which unfortunately bring the significance of the results into question. A third study, evaluating the effects of bread prepared with *Aloe* gel consumed twice daily for 3 mo, reported an incidental finding of decreased fasting and post-prandial blood glucose levels in the subjects diagnosed with diabetes[72]. Recently, Huseini *et al*[73], reported a study that evaluates the effects of *Aloe vera* gel in hyperlipidemic type 2 diabetes subjects and documented that Aloe gel significantly lowered fasting blood glucose, HbA1c, total and low-density lipoprotein cholesterol levels with no other side effects when administered twice a day at a dose of 300 mg for 8 wk[73].

Aloe chromone, a special type of polyphenol isolated from aloe leaves, and Aloe polysaccharides have well documented biological and anti-oxidation functions

Chromones isolated from various Aloe species have been reported to have diverse biological activity. A c-glycosyl chromone isolated from *Aloe barbadensis* demonstrates anti-inflammatory activity[74] and antioxidant activity similar to that of alpha-tocopherol based on a rat brain homogenates model[60]. The chemical components of *Aloe ferox* leaf gel were thoroughly analyzed with potent anti-oxidation properties reported and potential usages in alleviating symptoms and/or preventing diabetes were speculated[75]. Aloesin is a C-glucosylated 5-methylchromone with a potent anti-oxidation activity[76,77]. *In vitro*, aloesin is a strong inhibitor of tyrosinase activity[78] and up-regulates cyclin E-dependent kinase activity[79].

In a recent study where the phytochemical profile of *Aloe barbadensis* was investigated using colorimetric assays, triple quadrupole and time-of-flight mass spectrometry, focusing on phenolic secondary metabolites in the different leaf portions, the outer green rind that contains aloesin was identified as the most active in radical scavenging activity, than the inner parenchyma in stable radical DPPH test and oxygen radical absorption capacity (ORAC) assay. Further tests using isolated pure secondary metabolites confirmed as the 5-methylchromones aloesin were among the most active chromones[80].

Specifically, Aloesin was tested for ORAC relative to green tea extract and grape seed extract using the experimental procedures described in two publications[81,82]. It was found that Aloesin has an ORAC value (5331, 419 and 3221 for whole, 95% and 50% ORAC, respectively) that is much higher than the high purity polyphenols in green tea (2945, 481 and 1838 for whole, 95% and 50% ORAC, respectively) and grape seed extracts (3213, 312, 411 for whole, 95% and 50% ORAC, respectively). For comparison, the well-known antioxidants pure vitamin C and vitamin E have reported ORAC values of 2000 and 1162 μmol TE/g[83],

respectively.

Moreover, polysaccharides, the major constituents of *Aloe vera* gel, have been utilized for varieties of human disease and suggested for diabetes management, in part, because of their antioxidant activities. For instance, strong antioxidant activities have been reported for purified polysaccharides from *Aloe barbadensis* gel when tested in DPPH, hydroxyl and alkyl radical scavenging assays[84]. Similarly, in Aloe plant age and function related study, polysaccharides from three-years-old aloe extract were found showing the strongest radical scavenging activity (72.19%) which was significantly higher than that of synthetic antioxidants butylated hydroxytoluene (70.52%) and α-tocopherol (65.20%) at the same concentrations of 100 mg/L *via* DPPH assay[85]. Polysaccharides isolated from *Aloe vera* have also been found to possess high antioxidant efficiency as demonstrated with a decrease in the oxidative stress marker malondialdehyde and an increase in the hepatic non-enzymatic antioxidant GSH and enzymatic antioxidant SOD *in vivo* in chronic alcohol-induced hepatotoxicity in mice[86].

Therefore, these strong antioxidant activities of both Aloesin and aloe polysaccharides suggest their potential indications in diabetes to curve its devastating complications.

Aloesin can increase adiponectin production from adipocyte

Adiponectin - an adipocyte-derived plasma protein is exclusively produced by fat cells and its blood levels inversely correlates with insulin sensitivity and are thought to be predictive of susceptibility to type 2 diabetes[87]. It is believed that the key adipokin marker protein - adiponectin can modulate other glucose and fatty acid key metabolic pathways, improve directly and indirectly insulin resistance and glucose intolerance. The anti-atherosclerotic and anti-obesity effects of adiponectin have been well established. Recently Adiponectin has been discovered with suppression of high-glucose-induced ROS based on an *in vitro* model[88]. Therefore, finding a compound that can up regulate the production of adiponectin from adipocytes is a potential approach to managing the causal factor of diabetes and its complications.

Previously, we carried out a random screening of 2059 botanical extracts to identify natural substances that increase adiponectin production by adipocytes, *i.e.*, fat cells[89]. The initial screening yielded 139 positive hits. As a result of the subsequent verification assays and secondary screening, one active extract from leave exudates of *Aloe ferox*, designated as P0017, showed a consistent up modulating adiponectin level in the media. That led to the isolation and identification of Aloesin as the active component in the *Aloe ferox* extract. Aloesin tripled the adiponectin concentration in the culture media that was determined with an ELISA kit. In comparison, indomethacin at 10 μmol/L increased adiponectin production by 7-folds.

Gene expression study showed that a standardized composition containing Aloesin formulated with Aloe polysaccharides can down regulate fatty acid biosynthesis, and up regulated multiple key genes in the IR signaling cascade

It has also been shown that microarray analysis of gene expression modifications in white adipose tissue (WAT) and liver isolated from high fat diet induced pre-diabetes mice that were administered orally with Aloesin in *Aloe vera* gel powder (also known as Loesyn or UP780) to regulate fatty acid biosynthesis and up regulated multiple key genes in the IR signaling cascade. Specifically in liver, microarray analysis suggested that Loesyn modified multiple metabolic pathways for lipid metabolism such as decreased fatty acid biosynthesis, increased fatty acid binding proteins, decreased lipid uptake, and increased bile biosynthesis. These findings were also corroborated by quantitative polymerase chain reaction that showed Loesyn to cause coordinated increases in gene expression for multiple key genes in the IR signaling cascade such as up-regulation of IR (INSR), IRS1, and glucose transporter 4. The combined modifications to lipid metabolism in liver and insulin response in WAT suggested Aloesin delivered in *Aloe vera* gel powder can reduce the systemic oxidative stress by improving the glucose transportation and usage with enhanced insulin sensitivity and by reducing fatty acid synthesis[90].

Aloesin delivered as a pure compound or formulated within Aloe gel powders reduced fasting glucose, improved glucose tolerance and insulin sensitivity of diabetic animals

Impaired insulin sensitivity, glucose tolerance and metabolic disorders were induced in C57BL/6J mice by feeding the animals a high fat diet for 8 wk. The mice were then treated intraperitoneal with Aloesin at a dose of 100 mg/kg and a reference compound GW1929 at a dose of 5 mg/kg for 4 wk. Glucose and insulin tolerance tests were carried out on day 18 and day 24, respectively. Animals treated with Aloesin showed a significant improvement of glucose clearance and/or utilization in both tests compared to the vehicle treated animals. The insulin sensitizing activity of Aloesin was also further demonstrated by the ability of the compound in lowering the plasma insulin levels in the treated animals. The reference compound, GW1929 [the Active Pharmaceutical Ingredient for the Avandia™ (GSK) insulin sensitizer drug][91] induced a 50.2% reduction in plasma insulin compared to vehicle, as expected. Similarly, Aloesin showed 37.9% decreased in plasma insulin levels compared to that of the vehicle treated mice. In a subsequent study using high-fat diet induced diabetes mice, administered orally with chromone enriched aloe composition (UP780) at a dose of 200 mg/kg for 10 wk, showed a 30.3% decrease in fasting blood glucose levels and 32.2% reductions in plasma insulin with significant improvement in blood glucose clearance. Additionally, in *db/db* mice, the

composition also showed a 33.7% and 46.0% decrease in fasting triglyceride and plasma glucose levels after 10-wk oral treatment, respectively, when compared to vehicle.

Substantiating the above findings, administered orally at a dose of 2 g/kg, the composition UP780 has also showed reduced blood glucose and triglyceride, improved blood glucose clearance and plasma insulin level in alloxan induced insulin dependent mouse diabetes model[92].

In a double-blind, placebo controlled human clinical trial, Aloesin delivered within Aloe vera gel powder (referred as Loesyn) improved commonly monitored diabetic associated markers

Human clinical trial was carried out for Loesyn against placebo control by a third party University hospital for 8 wk following institutional review board approved protocol[93]. Subjects were given Loesyn at a dose of 500 mg capsules BID (*bis in die*) orally for a total daily dose of 1 g/d and equally matched in appearance placebo capsules for the duration of the study.

Inclusion criteria for pre-diabetes subjects were: fasting plasma glucose 100-125 mg/dL (5.55-6.94 mmol/L), waist circumference > 35 in (88.9 cm) females, > 40 in (101.6 cm) males, 2 h oral glucose tolerance test 149-199 mg/dL (8.27-11.05 mmol/L), HbA1c 5.0%-7.0%, Age > 25 years, No history of diabetes, or insulin or other diabetes medications, no cholesterol lowering or high dose antioxidants/anti-inflammatory medication or other concurrent dietary supplements, diet aids, weight loss programs, no other chronic conditions (heart disease, renal failure, or abnormal CBC).

A total of 30 subjects with impaired fasting glucose or impaired glucose tolerance were randomized to either placebo or Loesyn 500 mg BID for a period of 8 wk. After 8 wk of oral treatment, there were no significant changes in the placebo group on any of the parameters. On the other hand, indicators of improved glycemic control such as significant reductions in HbA1C as well as fasting glucose and fructosamine levels, were observed in the Loesyn treated subjects. The fasting glucose, HbA1C and fructosamine decreases were statistically significant ($P < 0.05$) for this group in comparison with placebo. Moreover, significant reduction in oxidative stress marker - urinary f2-isoprostanes was noted for subjects treated with Loesyn when compared to baseline.

There was no reduction of total cholesterol and triglycerides levels for subjects received either the composition or the placebo group. No side effects were reported or observed and there were no significant baseline differences between the composition and placebo groups. Similarly, no changes were observed on the safety evaluation parameters, cardiovascular variables (systolic and diastolic blood pressure), Complete Blood Count, chemistry profile, and liver function tests.

In a similar double-blind randomized controlled trial, a total of 136 subjects were recruited based on inclusion criteria such obesity (body mass index $\geqslant$ 25 kg/m^2) or abdominal obesity (waist circumference $\geqslant$ 90 cm for men or $\geqslant$ 85 cm for women), impaired fasting blood glucose FBG ($\geqslant$ 100 mg/dL) or impaired glucose tolerance (2-h oral glucose tolerance test ≥ 140 mg/dL), and subjects that would more likely to ensure a lifestyle modification to control blood sugar levels (FBG < 180 mg/dL and HbA1c < 8.0%). Such equally divided subjects received aloe vera gel complex containing Aloesin or Placebo at a dose of 700 mg/kg twice a day for 8 wk. Parameters were evaluated at baseline, week 4 and week 8. After 8 wk of repeated daily oral treatment, statistically significant reduction in body weight, body fat mass, and fasting blood glucose were observed for subjects with intervention. Homeostasis model of assessment - insulin resistance and serum insulin level were also found statistically significant at week 4 in these subjects compared to baseline[94].

CONCLUSION

Collectively, hyperglycemia in diabetes can induce imbalance of ROS by multiple metabolic pathways through increased flux of glucose *via* the polyol pathway, increased formation of AGEs and activation of their receptors, activation of PKC isoforms, over activity of hexosamine pathway, and decrease of antioxidant defenses. While hyperglycemia is a platform, ROS is the pivotal axis for diabetes and its complication development and progression. Appropriate glycemic management in associate with ROS balance control through antioxidants may counteract the complications of diabetes mellitus. Aloesin is a natural polyphenol originated from Aloe plants. Aloesin and/or aloe polysaccharides can reduce systemic oxidative stress by acting directly as a potent anti-oxidant and also indirectly by regulating the productions of adiponectin and gene expressions pathways related to insulin sensitivity, glucose transportation and fatty acid biosynthesis. The health benefits of supplementing a standardized composition containing Aloesin formulated with Aloe polysaccharides were demonstrated by animal studies on high fat diet and alloxan-induced as well as db/db diabetic mice models. Besides reducing fasting glucose, improving glucose tolerance, and enhancing insulin sensitivity, in a human clinical trial, the composition Loesyn also reduced oxidative stress marker in urine after 8 wk of oral supplements. Therefore, Loesyn formulated in foods could potentially be used either as over the counter or under the supervision of a physician for managing systemic oxidative stress of diabetes[95] and/or lowering blood glucose. This approach could likely make this inexpensive, safe and efficacious medical food product available quickly to the growing pre-diabetic and diabetic population worldwide.

ACKNOWLEDGMENTS

The authors would like to express their best gratitude

to Dr. Edward Cannon, Dr. Doug Bradley, Dr. Wenwen Ma, and Dr. Padma Abeysinghe, and Unigen team for their professional support for all the preclinical and clinical studies compiled in the manuscript pertaining to Unigen. The authors would like to extend their utmost gratitude to Mr. Bill Lee, the owner of Econet/Unigen, Inc., who supported all the preclinical and clinical studies described in this manuscript pertaining to Unigen.

REFERENCES

1 IDF Diabetes Atlas Sixth Edition Poster Update 2014. Key findings, 2014. [Accessed 2015 Mar 31]. Available from: URL: http://www.idf.org/diabetesatlas/update-2014

2 Insulin Resistance and Pre-diabetes. National Diabetes Information Clearinghouse. US Depar ent of Health and Human Services. National Institutes of Health. Available from: URL: http://www.diabetes.niddk.nih.gov

3 **American Diabetes Association**. Economic costs of diabetes in the US in 2012. *Diabetes Care* 2013; **36**: 1033-1046 [PMID: 23468086 DOI: 10.2337/dc12-2625]

4 **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

5 The Global Use of Medicines: Outlook through 2016. Report by the IMS Institute for Healthcare. Available from: URL: http://www.imshealth.com/deployedfiles/ims/Global/Content/Insights/IMS Institute for Healthcare Informatics/Global Use of Meds 2011/Medicines_Outlook_Through_2016_Report.pdf

6 **Evans JL**, Goldfine ID, Maddux BA, Grodsky GM. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocr Rev* 2002; **23**: 599-622 [PMID: 12372842]

7 **Domínguez C**, Ruiz E, Gussinye M, Carrascosa A. Oxidative stress at onset and in early stages of type 1 diabetes in children and adolescents. *Diabetes Care* 1998; **21**: 1736-1742 [PMID: 9773740]

8 **Matés JM**. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology* 2000; **153**: 83-104 [PMID: 11090949]

9 **Deneke SM**, Fanburg BL. Regulation of cellular glutathione. *Am J Physiol* 1989; **257**: L163-L173 [PMID: 2572174]

10 **Forsberg L**, de Faire U, Morgenstern R. Oxidative stress, human genetic variation, and disease. *Arch Biochem Biophys* 2001; **389**: 84-93 [PMID: 11370676]

11 **Nagy IZ**. On the true role of oxygen free radicals in the living state, aging, and degenerative disorders. *Ann N Y Acad Sci* 2001; **928**: 187-199 [PMID: 11795510]

12 **Blair AS**, Hajduch E, Litherland GJ, Hundal HS. Regulation of glucose transport and glycogen synthesis in L6 muscle cells during oxidative stress. Evidence for cross-talk between the insulin and SAPK2/p38 mitogen-activated protein kinase signaling pathways. *J Biol Chem* 1999; **274**: 36293-36299 [PMID: 10593919]

13 **Campbell RK**. Fate of the beta-cell in the pathophysiology of type 2 diabetes. *J Am Pharm Assoc* (2003) 2009; **49** Suppl 1: S10-S15 [PMID: 19801360 DOI: 10.1331/JAPhA.2009.09076]

14 **Evans JL**, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes* 2003; **52**: 1-8 [PMID: 12502486]

15 **Boucek P**. Advanced Diabetic Neuropathy: A Point of no Return? *Rev Diabet Stud* 2006; **3**: 143-150 [PMID: 17487338]

16 **Vincent AM**, Russell JW, Low P, Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocr Rev* 2004; **25**: 612-628 [PMID: 15294884]

17 **Askwith T**, Zeng W, Eggo MC, Stevens MJ. Oxidative stress and dysregulation of the taurine transporter in high-glucose-exposed human Schwann cells: implications for pathogenesis of diabetic neuropathy. *Am J Physiol Endocrinol Metab* 2009; **297**: E620-E628 [PMID: 19602579 DOI: 10.1152/ajpendo.00287.2009]

18 **Schmeichel AM**, Schmelzer JD, Low PA. Oxidative injury and apoptosis of dorsal root ganglion neurons in chronic experimental diabetic neuropathy. *Diabetes* 2003; **52**: 165-171 [PMID: 12502508]

19 **Ziegler D**, Sohr CG, Nourooz-Zadeh J. Oxidative stress and antioxidant defense in relation to the severity of diabetic polyneuropathy and cardiovascular autonomic neuropathy. *Diabetes Care* 2004; **27**: 2178-2183 [PMID: 15333481]

20 **Russell JW**, Berent-Spillson A, Vincent AM, Freimann CL, Sullivan KA, Feldman EL. Oxidative injury and neuropathy in diabetes and impaired glucose tolerance. *Neurobiol Dis* 2008; **30**: 420-429 [PMID: 18424057 DOI: 10.1016/j.nbd.2008.02.013]

21 **Goh SY**, Cooper ME. Clinical review: The role of advanced glycation end products in progression and complications of diabetes. *J Clin Endocrinol Metab* 2008; **93**: 1143-1152 [PMID: 18182449 DOI: 10.1210/jc.2007-1817]

22 **Vinik AI**. Advances in diabetes for the millennium: new treatments for diabetic neuropathies. *MedGenMed* 2004; **6**: 13 [PMID: 15647718]

23 **Vallianou N**, Evangelopoulos A, Koutalas P. Alpha-lipoic Acid and diabetic neuropathy. *Rev Diabet Stud* 2009; **6**: 230-236 [PMID: 20043035 DOI: 10.1900/RDS.2009.6.230]

24 **Singh U**, Jialal I. Alpha-lipoic acid supplementation and diabetes. *Nutr Rev* 2008; **66**: 646-657 [PMID: 19019027 DOI: 10.1111/j.1753-4887.2008.00118.x]

25 **Ueno Y**, Kizaki M, Nakagiri R, Kamiya T, Sumi H, Osawa T. Dietary glutathione protects rats from diabetic nephropathy and neuropathy. *J Nutr* 2002; **132**: 897-900 [PMID: 11983810]

26 **Jin HY**, Cha YS, Baek HS, Park TS. Neuroprotective effects of Vitis vinifera extract on prediabetic mice induced by a high-fat diet. *Korean J Intern Med* 2013; **28**: 579-586 [PMID: 24009454]

27 **Klein R**, Klein BE, Moss SE. Epidemiology of proliferative diabetic retinopathy. *Diabetes Care* 1992; **15**: 1875-1891 [PMID: 1464243]

28 **Cui Y**, Xu X, Bi H, Zhu Q, Wu J, Xia X, Qiushi Ren PC. Expression modification of uncoupling proteins and MnSOD in retinal endothelial cells and pericytes induced by high glucose: the role of reactive oxygen species in diabetic retinopathy. *Exp Eye Res* 2006; **83**: 807-816 [PMID: 16750827]

29 **Kowluru RA**, Kern TS, Engerman RL, Armstrong D. Abnormalities of retinal metabolism in diabetes or experimental galactosemia. III. Effects of antioxidants. *Diabetes* 1996; **45**: 1233-1237 [PMID: 8772728]

30 **Fiorentino TV**, Prioletta A, Zuo P, Folli F. Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. *Curr Pharm Des* 2013; **19**: 5695-5703 [PMID: 23448484]

31 **Ding Y**, Choi ME. Autophagy in diabetic nephropathy. *J Endocrinol* 2015; **224**: R15-R30 [PMID: 25349246 DOI: 10.1530/JOE-14-0437]

32 **Wang Z**, Choi ME. Autophagy in kidney health and disease. *Antioxid Redox Signal* 2014; **20**: 519-537 [PMID: 23642034]

33 **Ma T**, Zhu J, Chen X, Zha D, Singhal PC, Ding G. High glucose induces autophagy in podocytes. *Exp Cell Res* 2013; **319**: 779-789 [PMID: 23384600]

34 **Brownlee M**. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005; **54**: 1615-1625 [PMID: 15919781]

35 **Ceriello A**. New insights on oxidative stress and diabetic complications may lead to a "causal" antioxidant therapy. *Diabetes Care* 2003; **26**: 1589-1596 [PMID: 12716823]

36 **Lamb RE**, Goldstein BJ. Modulating an oxidative-inflammatory cascade: potential new treatment strategy for improving glucose metabolism, insulin resistance, and vascular function. *Int J Clin Pract* 2008; **62**: 1087-1095 [PMID: 18489578]

37 **Johansen JS**, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. *Cardiovasc Diabetol* 2005; **4**: 5 [PMID: 15862133]

38 **Benzie IF**. Evolution of antioxidant defence mechanisms. *Eur J*

Nutr 2000; **39**: 53-61 [PMID: 10918985]
39 **Pietta PG**. Flavonoids as antioxidants. *J Nat Prod* 2000; **63**: 1035-1042 [PMID: 10924197]
40 **Hung LM**, Su MJ, Chu WK, Chiao CW, Chan WF, Chen JK. The protective effect of resveratrols on ischaemia-reperfusion injuries of rat hearts is correlated with antioxidant efficacy. *Br J Pharmacol* 2002; **135**: 1627-1633 [PMID: 11934802]
41 **Takaoka M**, Ohkita M, Kobayashi Y, Yuba M, Matsumura Y. Protective effect of alpha-lipoic acid against ischaemic acute renal failure in rats. *Clin Exp Pharmacol Physiol* 2002; **29**: 189-194 [PMID: 11906481]
42 **Black HS**. Pro-oxidant and anti-oxidant mechanism(s) of BHT and beta-carotene in photocarcinogenesis. *Front Biosci* 2002; **7**: d1044-d1055 [PMID: 11897548]
43 **Sharman JE**, Gunaruwan P, Knez WL, Schmitt M, Marsh SA, Wilson GR, Cockcroft JR, Coombes JS. Alpha-lipoic acid does not acutely affect resistance and conduit artery function or oxidative stress in healthy men. *Br J Clin Pharmacol* 2004; **58**: 243-248 [PMID: 15327583]
44 **Roberts LJ**, Oates JA, Linton MF, Fazio S, Meador BP, Gross MD, Shyr Y, Morrow JD. The relationship between dose of vitamin E and suppression of oxidative stress in humans. *Free Radic Biol Med* 2007; **43**: 1388-1393 [PMID: 17936185]
45 **Maritim AC**, Sanders RA, Watkins JB. Diabetes, oxidative stress, and antioxidants: a review. *J Biochem Mol Toxicol* 2003; **17**: 24-38 [PMID: 12616644]
46 **Garcia-Bailo B**, El-Sohemy A, Haddad PS, Arora P, Benzaied F, Karmali M, Badawi A. Vitamins D, C, and E in the prevention of type 2 diabetes mellitus: modulation of inflammation and oxidative stress. *Biologics* 2011; **5**: 7-19 [PMID: 21383912 DOI: 10.2147/BTT.S14417]
47 **Chang YC**, Chuang LM. The role of oxidative stress in the pathogenesis of type 2 diabetes: from molecular mechanism to clinical implication. *Am J Transl Res* 2010; **2**: 316-331 [PMID: 20589170]
48 **Dandona P**, Ghanim H, Chaudhuri A, Dhindsa S, Kim SS. Macronutrient intake induces oxidative and inflammatory stress: potential relevance to atherosclerosis and insulin resistance. *Exp Mol Med* 2010; **42**: 245-253 [PMID: 20200475]
49 **Valtueña S**, Pellegrini N, Franzini L, Bianchi MA, Ardigò D, Del Rio D, Piatti P, Scazzina F, Zavaroni I, Brighenti F. Food selection based on total antioxidant capacity can modify antioxidant intake, systemic inflammation, and liver function without altering markers of oxidative stress. *Am J Clin Nutr* 2008; **87**: 1290-1297 [PMID: 18469252]
50 **Scalbert A**, Williamson G. Dietary intake and bioavailability of polyphenols. *J Nutr* 2000; **130**: 2073S-2085S [PMID: 10917926]
51 **Scalbert A**, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. *Am J Clin Nutr* 2005; **81**: 215S-217S [PMID: 15640483]
52 **Manach C**, Scalbert A, Morand C, Rémésy C, Jiménez L. Polyphenols: food sources and bioavailability. *Am J Clin Nutr* 2004; **79**: 727-747 [PMID: 15113710]
53 **Medić-Sarić M**, Rastija V, Bojić M, Males Z. From functional food to medicinal product: systematic approach in analysis of polyphenolics from propolis and wine. *Nutr J* 2009; **8**: 33 [PMID: 19624827 DOI: 10.1186/1475-2891-8-33]
54 **Cheynier V**. Polyphenols in foods are more complex than often thought. *Am J Clin Nutr* 2005; **81**: 223S-229S [PMID: 15640485]
55 **Tarascou I**, Souquet JM, Mazauric JP, Carrillo S, Coq S, Canon F, Fulcrand H, Cheynier V. The hidden face of food phenolic composition. *Arch Biochem Biophys* 2010; **501**: 16-22 [PMID: 20363210 DOI: 10.1016/j.abb.2010.03.018]
56 **Nettleton JA**, Harnack LJ, Scrafford CG, Mink PJ, Barraj LM, Jacobs DR. Dietary flavonoids and flavonoid-rich foods are not associated with risk of type 2 diabetes in postmenopausal women. *J Nutr* 2006; **136**: 3039-3045 [PMID: 17116717]
57 **Song Y**, Manson JE, Buring JE, Sesso HD, Liu S. Associations of dietary flavonoids with risk of type 2 diabetes, and markers of insulin resistance and systemic inflammation in women: a prospective study and cross-sectional analysis. *J Am Coll Nutr* 2005; **24**: 376-384 [PMID: 16192263]
58 **Natow AJ**. Aloe vera, fiction or fact. *Cutis* 1986; **37**: 106, 108 [PMID: 3956253]
59 **Reynolds T**, Dweck AC. Aloe vera leaf gel: a review update. *J Ethnopharmacol* 1999; **68**: 3-37 [PMID: 10624859]
60 **Lee KY**, Weintraub ST, Yu BP. Isolation and identification of a phenolic antioxidant from Aloe barbadensis. *Free Radic Biol Med* 2000; **28**: 261-265 [PMID: 11281293]
61 **Ajabnoor MA**. Effect of aloes on blood glucose levels in normal and alloxan diabetic mice. *J Ethnopharmacol* 1990; **28**: 215-220 [PMID: 2109811]
62 **Ghannam N**, Kingston M, Al-Meshaal IA, Tariq M, Parman NS, Woodhouse N. The antidiabetic activity of aloes: preliminary clinical and experimental observations. *Horm Res* 1986; **24**: 288-294 [PMID: 3096865]
63 **Rajasekaran S**, Ravi K, Sivagnanam K, Subramanian S. Beneficial effects of aloe vera leaf gel extract on lipid profile status in rats with streptozotocin diabetes. *Clin Exp Pharmacol Physiol* 2006; **33**: 232-237 [PMID: 16487267]
64 **al-Awadi F**, Fatania H, Shamte U. The effect of a plants mixture extract on liver gluconeogenesis in streptozotocin induced diabetic rats. *Diabetes Res* 1991; **18**: 163-168 [PMID: 1842751]
65 **Parihar MS**, Chaudhary M, Shetty R, Hemnani T. Susceptibility of hippocampus and cerebral cortex to oxidative damage in streptozotocin treated mice: prevention by extracts of Withania somnifera and Aloe vera. *J Clin Neurosci* 2004; **11**: 397-402 [PMID: 15080956]
66 **Bolkent S**, Akev N, Ozsoy N, Sengezer-Inceli M, Can A, Alper O, Yanardag R. Effect of Aloe vera (L.) Burm. fil. leaf gel and pulp extracts on kidney in type-II diabetic rat models. *Indian J Exp Biol* 2004; **42**: 48-52 [PMID: 15274480]
67 **Can A**, Akev N, Ozsoy N, Bolkent S, Arda BP, Yanardag R, Okyar A. Effect of Aloe vera leaf gel and pulp extracts on the liver in type-II diabetic rat models. *Biol Pharm Bull* 2004; **27**: 694-698 [PMID: 15133247]
68 **Yeh GY**, Eisenberg DM, Kaptchuk TJ, Phillips RS. Systematic review of herbs and dietary supplements for glycemic control in diabetes. *Diabetes Care* 2003; **26**: 1277-1294 [PMID: 12663610]
69 **Vogler BK**, Ernst E. Aloe vera: a systematic review of its clinical effectiveness. *Br J Gen Pract* 1999; **49**: 823-828 [PMID: 10885091]
70 **Yongchaiyudha S**, Rungpitarangsi V, Bunyapraphatsara N, Chokechaijaroenporn O. Antidiabetic activity of Aloe vera L. juice. I. Clinical trial in new cases of diabetes mellitus. *Phytomedicine* 1996; **3**: 241-243 [PMID: 23195077 DOI: 10.1016/S0944-7113(96)80060-2]
71 **Bunyapraphatsara N**, Yongchaiyudha S, Rungpitarangsi V, Chokechaijaroenporn O. Antidiabetic activity of Aloe vera L. juice II. Clinical trial in diabetes mellitus patients in combination with glibenclamide. *Phytomedicine* 1996; **3**: 245-248 [PMID: 23195078 DOI: 10.1016/S0944-7113(96)80061-4]
72 **Agarwal OP**. Prevention of atheromatous heart disease. *Angiology* 1985; **36**: 485-492 [PMID: 2864002]
73 **Huseini HF**, Kianbakht S, Hajiaghaee R, Dabaghian FH. Anti-hyperglycemic and anti-hypercholesterolemic effects of Aloe vera leaf gel in hyperlipidemic type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. *Planta Med* 2012; **78**: 311-316 [PMID: 22198821 DOI: 10.1055/s-0031-1280474]
74 **Hutter JA**, Salman M, Stavinoha WB, Satsangi N, Williams RF, Streeper RT, Weintraub ST. Antiinflammatory C-glucosyl chromone from Aloe barbadensis. *J Nat Prod* 1996; **59**: 541-543 [PMID: 8778246]
75 **Loots du T**, van der Westhuizen FH, Botes L. Aloe ferox leaf gel phytochemical content, antioxidant capacity, and possible health benefits. *J Agric Food Chem* 2007; **55**: 6891-6896 [PMID: 17661490]
76 **Jia Q**, Farrow TM. 7-Hydroxy Chromones as Potent Anti-oxidant. USA Patent. 7, 678, 772. 2010 March 16
77 **Holdsworth DK**. Chromones in aloe species. I. Aloesin--a C-glucosyl-7-hydroxychromone. *Planta Med* 1971; **19**: 322-325

[PMID: 5573544]
78 **Yagi A**, Kanbara T, Morinobu N. Inhibition of mushroom-tyrosinase by aloe extract. *Planta Med* 1987; **53**: 515-517 [PMID: 17269093]
79 **Lee KY**, Park JH, Chung MH, Park YI, Kim KW, Lee YJ, Lee SK. Aloesin up-regulates cyclin E/CDK2 kinase activity via inducing the protein levels of cyclin E, CDK2, and CDC25A in SK-HEP-1 cells. *Biochem Mol Biol Int* 1997; **41**: 285-292 [PMID: 9063568]
80 **Lucini L**, Pellizzoni M, Pellegrino R, Molinari GP, Colla G. Phytochemical constituents and in vitro radical scavenging activity of different Aloe species. *Food Chem* 2015; **170**: 501-507 [PMID: 25306376 DOI: 10.1016/j.foodchem.2014.08.034]
81 **Ghiselli A**, Serafini M, Ferro-Luzzi A. New approaches for measuring plasma or serum antioxidant capacity: a methodological note. *Free Radic Biol Med* 1994; **16**: 135-137 [PMID: 8299989]
82 **Prior RL**, Cao G. Antioxidant capacity and polyphenolic components of teas: implications for altering in vivo antioxidant status. *Proc Soc Exp Biol Med* 1999; **220**: 255-261 [PMID: 10202399]
83 **Ou B**, Huang D, Hampsch-Woodill M, Flanagan JA. When east meets west: the relationship between yin-yang and antioxidation-oxidation. *FASEB J* 2003; **17**: 127-129 [PMID: 12554690]
84 **Kang MC**, Kim SY, Kim YT, Kim EA, Lee SH, Ko SC, Wijesinghe WA, Samarakoon KW, Kim YS, Cho JH, Jang HS, Jeon YJ. In vitro and in vivo antioxidant activities of polysaccharide purified from aloe vera (Aloe barbadensis) gel. *Carbohydr Polym* 2014; **99**: 365-371 [PMID: 24274519 DOI: 10.1016/j.carbpol.2013.07.091]
85 **Hu Y**, Xu J, Hu Q. Evaluation of antioxidant potential of aloe vera (Aloe barbadensis miller) extracts. *J Agric Food Chem* 2003; **51**: 7788-7791 [PMID: 14664546]
86 **Cui Y**, Ye Q, Wang H, Li Y, Yao W, Qian H. Hepatoprotective potential of Aloe vera polysaccharides against chronic alcohol-induced hepatotoxicity in mice. *J Sci Food Agric* 2014; **94**: 1764-1771 [PMID: 24272968 DOI: 10.1002/jsfa.6489]
87 **Yamauchi T**, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; **7**: 941-946 [PMID: 11479627]
88 **Ouedraogo R**, Wu X, Xu SQ, Fuchsel L, Motoshima H, Mahadev K, Hough K, Scalia R, Goldstein BJ. Adiponectin suppression of high-glucose-induced reactive oxygen species in vascular endothelial cells: evidence for involvement of a cAMP signaling pathway. *Diabetes* 2006; **55**: 1840-1846 [PMID: 16731851]
89 **Yimam M**, Zhao J, Tseng-Crank J, Brownell L, Devaraj S, Jialal R, Jialal I, Rockwood J, Ma W, Padmapriya A, Jia Q. A novel standardized chromone extract (UP780) from Aloe regulated adiponectin production and improved insulin resistance. 7th Joint Meeting of GA, AFERP, ASP, PSE & SIF Abstracts. *Planta Med* 2008; **74**: SL78 [DOI: 10.1055/s-0028-1083958]
90 **Tseng-Crank J**, Do SG, Corneliusen B, Hertel C, Homan J, Yimam M, Zhao J, Jia Q. UP780, a Chromone-Enriched Aloe Composition, Enhances Adipose Insulin Receptor Signaling and Decreases Liver Lipid Biosynthesis. *Open J Genet* 2013; **3**: 9-86
91 **Yimam M**, Zhao J, Corneliusen B, Pantier M, Brownell LA, Jia Q. UP780, a chromone-enriched aloe composition improves insulin sensitivity. *Metab Syndr Relat Disord* 2013; **11**: 267-275 [PMID: 23573999 DOI: 10.1089/met.2012.0135]
92 **Yimam M**, Zhao J, Corneliusen B, Pantier M, Brownell L, Jia Q. Blood glucose lowering activity of aloe based composition, UP780, in alloxan induced insulin dependent mouse diabetes model. *Diabetol Metab Syndr* 2014; **6**: 61 [PMID: 24891878 DOI: 10.1186/1758-5996-6-61]
93 **Devaraj S**, Yimam M, Brownell LA, Jialal I, Singh S, Jia Q. Effects of Aloe vera supplementation in subjects with prediabetes/metabolic syndrome. *Metab Syndr Relat Disord* 2013; **11**: 35-40 [PMID: 23035844 DOI: 10.1089/met.2012.0066]
94 **Choi HC**, Kim SJ, Son KY, Oh BJ, Cho BL. Metabolic effects of aloe vera gel complex in obese prediabetes and early non-treated diabetic patients: randomized controlled trial. *Nutrition* 2013; **29**: 1110-1114 [PMID: 23735317 DOI: 10.1016/j.nut.2013.02.015]
95 **Zhao JF**, Tseng-Crank J, Yimam M, Jia Q. Chromones as Therapeutic Agents. US Patent. 8852657. 2014 October 7

P- Reviewer: Charoenphandhu N, Hill DJ, Nakhoul FM, Ozdemir S
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v6.i9.1108

World J Diabetes 2015 August 10; 6(9): 1108-1112
ISSN 1948-9358 (online)

Limited joint mobility syndrome in diabetes mellitus: A minireview

Esther G Gerrits, Gijs W Landman, Leonie Nijenhuis-Rosien, Henk J Bilo

Esther G Gerrits, Department of Internal Medicine, Maastricht University Medical Center, 6229 HX Maastricht, The Netherlands

Gijs W Landman, Leonie Nijenhuis-Rosien, Henk J Bilo, Diabetes Centre, Isala, 8025 AB Zwolle, The Netherlands

Gijs W Landman, Department of Internal Medicine, Gelre Hospital, 7334 DZ Apeldoorn, The Netherlands

Leonie Nijenhuis-Rosien, Innofeet Voetencentrum Nijenhuis, 8013 NA Zwolle, The Netherlands

Henk J Bilo, Department of Internal Medicine, Isala, 8025 AB Zwolle, The Netherlands

Author contributions: Gerrits EG designed and wrote the manuscript; Landman GW, Nijenhuis-Rosien L and Bilo HJ contributed equally to the writing of the manuscript.

Conflict-of-interest statement: The authors have no conflicts-of-interest.

Correspondence to: Esther G Gerrits, MD, PhD, Department of Internal Medicine, Maastricht University Medical Center, P. Debyelaan 25, 6229 HX Maastricht, The Netherlands. esther.gerrits@mumc.nl
Telephone: +31-43-3877005
Fax: +31-43-3875006

Received: February 22, 2015
Peer-review started: March 9, 2015
First decision: June 3, 2015
Revised: June 16, 2015
Accepted: July 24, 2015
Article in press: July 27, 2015

Published online: August 10, 2015

Abstract

Limited joint mobility syndrome (LJMS) or diabetic cheiroarthropathy is a long term complication of diabetes mellitus. The diagnosis of LJMS is based on clinical features: progression of painless stiffness of hands and fingers, fixed flexion contractures of the small hand and foot joints, impairment of fine motion and impaired grip strength in the hands. As the syndrome progresses, it can also affect other joints. It is important to properly diagnose such a complication as LJMS. Moreover, it is important to diagnose LJMS because it is known that the presence of LJMS is associated with micro- and macrovascular complications of diabetes. Due to the lack of curative treatment options, the suggested method to prevent or decelerate the development of LJMS is improving or maintaining good glycemic control. Daily stretching excercises of joints aim to prevent or delay progression of joint stiffness, may reduce the risk of inadvertent falls and will add to maintain quality of life.

Key words: Diabetic cheiroarthropathy; Limited joint mobility; Diabetes mellitus; Joint stiffness; Advanced glycation endproducts

Core tip: "Limited joint mobility syndrome in diabetes mellitus: A minireview" is an article about limited joint mobility syndrome in diabetes mellitus that is an underreported complication, associated with micro and macrovascular complications. From a clinical perspective, a good glycemic control and daily exercising are the main and the base of prevention. Treatment options include symptomatic therapies and surgical correction. Medical treatment targeting the

formation of glycosylated end products accumulating on collagen and other connective tissues are unsuccessful for this complication. This mini-review analyzes all the aspects of a forgotten complication of diabetes mellitus.

Gerrits EG, Landman GW, Nijenhuis-Rosien L, Bilo HJ. Limited joint mobility syndrome in diabetes mellitus: A minireview. *World J Diabetes* 2015; 6(9): 1108-1112 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i9/1108.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i9.1108

INTRODUCTION

Musculoskeletal disorders such as Achilles tendon pathology, trigger finger, Dupuytren, limited joint mobility syndrome (LJMS), carpal tunnel syndrome, frozen shoulder and plantar fasciitis have been found to occur more often in subjects with diabetes compared to those without diabetes[1-5]. With the increasing number of patients known with diabetes and - consequently - an increase in incidence and prevalence of diabetes related complications along with increasing age of these patient group, it is important to pay attention to the topic of musculoskeletal disorders in order to recognize and diagnose these disorders in clinical practice as early as possible. LJMS is one of the musculoskeletal disorders and is rather underexposed and underdiagnosed compared to the well-known micro- and macrovascular complications of diabetes. Due to their relative relationship to mortality, more attention is paid towards the complications of diabetes such as nephropathy, neuropathy and cardiovascular disease. Less attention is paid to LJMS, although it is associated with neuropathy and other microvascular complications and it can influence patients' health-related quality of life quite dramatically[1,4-9]. In this mini-review, we will exclusively focus on LJMS as a musculoskeletal complication of diabetes. It provides an overview of the pathofysiology, the importance of diagnosing LJMS, the practical implications of the diagnosis and future expectations on this topic.

LJMS

Epidemiology

Stiff hands in long-term diabetes has been described for the first time by Lundbaek[10] in 1957. Less reports have been published about LJMS until 1974, when Rosenbloom *et al*[11] exhibited renewed interest in this syndrome. Joint stiffness and contractures were described as a common feature in children with type 1 diabetes mellitus[11-13]. Currently, we define LJMS as a long term complication of diabetes mellitus, but it can also develop in patients without diabetes. The reported prevalence in diabetes mellitus apparently varies between 8%-58%, depending on the different diabetes patients cohorts and the applied definitions of LJMS[5-9,12,14-16]. The prevalence of LJMS in subjects without DM is difficult to estimate and may vary between 4%-26%[2,4,5,9]. Generally, no clear gender or racial preferences have been found in the development of LJMS in diabetes.

Symptomatology and diagnosis

LJMS of the hands and fingers, also called cheiroarthropathy, is characterized by several clinical features which enhance painless stiffness of hands and fingers, fixed flexion contractures of the small hand joints, impairment of fine motion and impaired grip strength. Ultimately, these features will result in the impairment of joint mobility, especially of the small joints of the hands and may become painful. The "prayer sign" and the "tabletop sign" are clinical tests strongly supporting the diagnosis, which can only be used in the absence of previous hand injury or hand surgery[17]. Under normal conditions, both hands will have contact for the total opposing hand surface parts, when the hands are pressed flat to each other, as making a "prayer sign". If this proves to be impossible, it means there are flexion contractures of the fingers and the sign is considered positive. With the "tabletop sign" one has to put the hands flat on the table with the fore arm in a 90 degree angle. If one hand doesn't make contact with the table at one spot, it means that there are contractures of the small hand joints suggesting the test positive.

Natural course: Besides joints involvement of hands, LJMS can also occur in the small joints of the feet and in the long term progression of disease can also result in impairment of other joints such as the shoulder, hip, ankle, spine and all other joints. Consequently, on the long term, LJMS might increase the risk of falling[18]. A limb threatening situation might occur when the impairment of mobility of toes and feet joints is seen in combination with the presence of neuropathy. The combination can lead to serious plantary pressure points, which translates into a great risk for diabetic foot ulcer[19-21]. When peripheral arterial disease is present, this might even result into an enhanced amputation risk. Conceivably, all these features and complications of LJMS can be accompanied by a significant reduced quality of life.

Differential diagnosis

Sometimes, LJMS is difficult to distinguish from other joint complaints in diabetes patients. Certain musculoskeletal conditions occur more frequently in diabetes patients compared to the general population which include Dupuytren, tenosynovitis and palmar/plantar fasciitis. Complex regional pain syndrome and scleroderma are also part of the differential diagnosis of LJM. The specific clinical features of each different disorder with or without supplementary laboratory and radio- or ultrasonographic evaluation confirm the diagnosis[22-24].

One should keep in mind that any supplementary diagnostic evaluation is quite unspecific, so the diagnosis of limitation of joint mobility mainly relies on the clinical features.

Considering a prevalence of up to 50% and the LJMS accompanied microvascular and limb threatening complications, screening for LJMS in diabetes patients is important, and has to be part of the annual check up or more often when indicated.

PATHOGENESIS

The apparently higher prevalence of LJMS in subjects with diabetes compared to nondiabetic subjects is assuming that there is a correlation between diabetes mellitus and LJMS, but good literature to support this correlation is lacking. As the presence of LJMS is associated with nephropathy, retinopathy and neuropathy, it is not only important to diagnose LJMS *per se*, but also because it can be an early warning signal of the possible presence of one or more of the microvascular complications[1,4-9]. In some cases it might be the first feature of tissue damage in diabetes which should alert physicians to actively screen or search for the presence of microvascular complications as well.

In general, the chances to develop LJMS are associated with age, diabetes duration and degree of glycemic control[1,4-6,9,14]. Theoretically, good glycemic control should diminish the risk of LJMS in an identical fashion as the development of other diabetic complications. Eventually, a combination of factors will contribute to the development and progression of diabetic complications including LJMS.

Besides a variable genetic susceptibility, high oxidative stress levels seem to be one of the factors involved. Intracellular hyperglycemia will cause high levels of oxidative stress and the formation of advanced glycation endproducts (AGEs). These AGEs are damaging glycosylation products, nonezymatically formed under circumstances of hyperglycemic and oxidative stress. In such an unfavourable environment, increased production of reactive oxygen species will be induced that can initiate the inflammatory cascade leading to the production of several cytokines and growth factors causing the hyperglycemia-induced cellular damage[25,26].

Furthermore, besides their damaging effects on the vascular endothelium, these accelerated formated AGEs also form cross-links with long-lived proteins such as skin collagen, tendons and ligaments altering their biological structure and function[27-29]. Collagen has a long half life, which means that collagen degradation will take a long time: for more than ten years. Therefore, the AGE-cross-links to collagen will extensively accumulate in the skin, tendons and ligaments and are considered to play an important role in the development of LJMS.

Genetic susceptibility in combination with other factors such as a hyperglycemic and highly oxidative stress environment will add to the development of LJMS.

THERAPEUTIC OPTIONS

LJMS seems to be an irreversible disorder with no specific curative treatment options. There are no drugs available which directly target LJMS. Only symptomatic therapy, such as analgesics, non-steroidal anti-inflammatory drugs or local corticosteroid injections can be given as a relief and in case of tendinitis or flexor tendon contractures. Surgery is indicated in case of severe contractures. Exercising, which include daily stretching exercises of the palm of the hand and sole of the foot, will also help to prevent or further delay the development of progressive joint stiffness in case of limited joint mobility[30]. In case of limited lower limb joint mobility with or without the presence of neuropathy, professional foot care and rocker bottom shoes are indispensible in order to prevent the developent of diabetic foot ulcers[31].

In general, as LJMS is associated with glycemic control and diabetes duration, just like all other diabetic complications, the best way to prevent LJMS is to strive for good glycemic control from the onset of diabetes diagnosis.

NOVEL STRATEGIES

During the past 20 years, research has been performed to find efficient agents with AGE inhibitory properties without toxicity, meant for safe application in humans. Targeting AGE cross-links with alagebrium (ALT-711) in experimental settings have clearly shown beneficial effects, but in human trials there seems to be a safety concern and alagebrium still has to be proven to be beneficial[32-34]. Aminoguanidine with a preventive effect on the formation and accumulation of AGEs in experimental studies, but not recommended for daily clinical use because of safety concerns and lack of evidence in human[34,35]. Anti-oxidant agents with specific AGE-inhibiting effects (*e.g.*, pyridoxamine, benfotiamine) have shown beneficial effects in animal models, but still have to be proven as an effective therapy in human[34].

With all these unsuccesfull strategies, newly developed targeted drugs are needed in order to prevent or delay the onset of LJMS.

CONCLUSION

LJMS is an underreported complication of diabetes, along and associated with micro- and macrovascular complications, which should be assessed during the annual check up of diabetes care. From a practical perspective, both a good glycemic control and daily excercising are the main and actually only pillars of prevention. Treatment options include symptomatic therapies and surgical correction. Medical treatment targeting the

formation of glycosylated endproducts accumulating on collagen and other connective tissues that are said to be responsible for the development of LJMS, have so far proved to be unsuccesfull. Newly developed targeted drugs are needed in order to prevent or delay the onset of LJMS, to reduce the risk of inadvertent falls and to maintain quality of life of subjects with diabetes.

REFERENCES

1 **Pandey A**, Usman K, Reddy H, Gutch M, Jain N, Qidwai S. Prevalence of hand disorders in type 2 diabetes mellitus and its correlation with microvascular complications. *Ann Med Health Sci Res* 2013; **3**: 349-354 [PMID: 24116312 DOI: 10.4103/2141-9248.117942]

2 **Smith LL**, Burnet SP, McNeil JD. Musculoskeletal manifestations of diabetes mellitus. *Br J Sports Med* 2003; **37**: 30-35 [PMID: 12547740 DOI: 10.1136/bjsm.37.1.30]

3 **Cagliero E**, Apruzzese W, Perlmutter GS, Nathan DM. Musculoskeletal disorders of the hand and shoulder in patients with diabetes mellitus. *Am J Med* 2002; **112**: 487-490 [PMID: 11959060 DOI: 10.1016/S0002-9343(02)01045-8]

4 **Pal B**, Anderson J, Dick WC, Griffiths ID. Limitation of joint mobility and shoulder capsulitis in insulin- and non-insulin-dependent diabetes mellitus. *Br J Rheumatol* 1986; **25**: 147-151 [PMID: 3708230]

5 **Starkman HS**, Gleason RE, Rand LI, Miller DE, Soeldner JS. Limited joint mobility (LJM) of the hand in patients with diabetes mellitus: relation to chronic complications. *Ann Rheum Dis* 1986; **45**: 130-135 [PMID: 3947142]

6 **Jennings AM**, Milner PC, Ward JD. Hand abnormalities are associated with the complications of diabetes in type 2 diabetes. *Diabet Med* 1989; **6**: 43-47 [PMID: 2522373 DOI: 10.1111/j.1464-5491.1989.tb01137.x]

7 **Rosenbloom AL**, Silverstein JH, Lezotte DC, Richardson K, McCallum M. Limited joint mobility in childhood diabetes mellitus indicates increased risk for microvascular disease. *N Engl J Med* 1981; **305**: 191-194 [PMID: 7242598 DOI: 10.1056/NEJM198107233050403]

8 **Jacobson AM**, Braffett BH, Cleary PA, Gubitosi-Klug RA, Larkin ME. The long-term effects of type 1 diabetes treatment and complications on health-related quality of life: a 23-year follow-up of the Diabetes Control and Complications/Epidemiology of Diabetes Interventions and Complications cohort. *Diabetes Care* 2013; **36**: 3131-3138 [PMID: 23835693 DOI: 10.2337/dc12-2109]

9 **Arkkila PE**, Kantola IM, Viikari JS. Limited joint mobility in non-insulin-dependent diabetic (NIDDM) patients: correlation to control of diabetes, atherosclerotic vascular disease, and other diabetic complications. *J Diabetes Complications* 1994; **11**: 208-217 [PMID: 9201597 DOI: 10.1016/S1056-8727(96)00038-4]

10 **Lundbaek K**. Stiff hands in long-term diabetes. *Acta Med Scand* 1957; **158**: 447-451 [PMID: 13469265 DOI: 10.1111/j.0954-6820.1957.tb15511.x]

11 **Rosenbloom AL**, Frias JL. Diabetes mellitus, short stature and joint stiffness - a new syndrome. *Clin Res* 1974; **22**: 92A

12 **Grgic A**, Rosenbloom AL, Weber FT, Giordano B, Malone JI, Shuster JJ. Joint contracture--common manifestation of childhood diabetes mellitus. *J Pediatr* 1976; **88**: 584-588 [PMID: 1255316]

13 **Benedetti A**, Noacco C. Juvenile diabetic cheiroarthropathy. *Acta Diabetol Lat* 1976; **13**: 54-67 [PMID: 970070]

14 **Gamstedt A**, Holm-Glad J, Ohlson CG, Sundström M. Hand abnormalities are strongly associated with the duration of diabetes mellitus. *J Intern Med* 1993; **234**: 189-193 [PMID: 8340742 DOI: 10.1111/j.1365-2796.1993.tb00729.x]

15 **Sukenik S**, Weitzman S, Buskila D, Eyal A, Gross J, Horowitz J. Limited joint mobility and other rheumatological manifestations in diabetic patients. *Diabete Metab* 1987; **13**: 187-192 [PMID: 3609420]

16 **Traisman HS**, Traisman ES, Marr TJ, Wise J. Joint contractures in patients with juvenile diabetes and their siblings. *Diabetes Care* 1978; **1**: 360-361 [PMID: 729450 DOI: 10.2337/diacare.1.6.360]

17 **Sauseng S**, Kästenbauer T, Irsigler K. Limited joint mobility in selected hand and foot joints in patients with type 1 diabetes mellitus: a methodology comparison. *Diabetes Nutr Metab* 2002; **15**: 1-6 [PMID: 11942733]

18 **López-Martín I**, Benito Ortiz L, Rodríguez-Borlado B, Cano Langreo M, García-Martínez FJ, Martín Rodríguez MF. [Association between limited joint mobility syndrome and risk of accidental falls in diabetic patients]. *Semergen* 2015; **41**: 70-75 [PMID: 24906788 DOI: 10.1016/j.semerg.2014.03.007]

19 **Delbridge L**, Perry P, Marr S, Arnold N, Yue DK, Turtle JR, Reeve TS. Limited joint mobility in the diabetic foot: relationship to neuropathic ulceration. *Diabet Med* 1988; **5**: 333-337 [PMID: 2968881]

20 **Fernando DJ**, Masson EA, Veves A, Boulton AJ. Relationship of limited joint mobility to abnormal foot pressures and diabetic foot ulceration. *Diabetes Care* 1991; **14**: 8-11 [PMID: 1991444 DOI: 10.2337/diacare.14.1.8]

21 **Zimny S**, Schatz H, Pfohl M. The role of limited joint mobility in diabetic patients with an at-risk foot. *Diabetes Care* 2004; **27**: 942-946 [PMID: 15047653 DOI: 10.2337/diacare.27.4.942]

22 **Ismail AA**, Dasgupta B, Tanqueray AB, Hamblin JJ. Ultrasonographic features of diabetic cheiroarthropathy. *Br J Rheumatol* 1996; **35**: 676-679 [PMID: 8670603 DOI: 10.1093/rheumatology/35.7.676]

23 **Duffin AC**, Lam A, Kidd R, Chan AK, Donaghue KC. Ultrasonography of plantar soft tissues thickness in young people with diabetes. *Diabet Med* 2002; **19**: 1009-1013 [PMID: 12647842 DOI: 10.1046/j.1464-5491.2002.00850.x]

24 **Khanna G**, Ferguson P. MRI of diabetic cheiroarthropathy. *AJR Am J Roentgenol* 2007; **188**: W94-W95 [PMID: 17179335 DOI: 10.2214/AJR.06.0672]

25 **Brownlee M**. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005; **54**: 1615-1625 [PMID: 15919781 DOI: 10.2337/diabetes.54.6.1615]

26 **Franco R**, Sánchez-Olea R, Reyes-Reyes EM, Panayiotidis MI. Environmental toxicity, oxidative stress and apoptosis: ménage à trois. *Mutat Res* 2009; **674**: 3-22 [PMID: 19114126 DOI: 10.1016/j.mrgentox.2008.11.012]

27 **Goldin A**, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: sparking the development of diabetic vascular injury. *Circulation* 2006; **114**: 597-605 [PMID: 16894049 DOI: 10.1161/circulationAHA.106.621854]

28 **Reddy GK**. Cross-linking in collagen by nonenzymatic glycation increases the matrix stiffness in rabbit achilles tendon. *Exp Diabesity Res* 2004; **5**: 143-153 [PMID: 15203885 DOI: 10.1080/15438600490277860]

29 **Reiser KM**. Nonenzymatic glycation of collagen in aging and diabetes. *Proc Soc Exp Biol Med* 1991; **196**: 17-29 [PMID: 1984239]

30 **Francia P**, Gulisano M, Anichini R, Seghieri G. Diabetic foot and exercise therapy: step by step the role of rigid posture and biomechanics treatment. *Curr Diabetes Rev* 2014; **10**: 86-99 [PMID: 24807636]

31 **Lawall H**, Diehm C. [Diabetic foot syndrome from the perspective of angiology and diabetology]. *Orthopade* 2009; **38**: 1149-1159 [PMID: 19949939 DOI: 10.1007/s00132-009-1501-z]

32 **Kim JB**, Song BW, Park S, Hwang KC, Cha BS, Jang Y, Lee HC, Lee MH. Alagebrium chloride, a novel advanced glycation end-product cross linkage breaker, inhibits neointimal proliferation in a diabetic rat carotid balloon injury model. *Korean Circ J* 2010; **40**: 520-526 [PMID: 21088756 DOI: 10.4070/kcj.2010.40.10.520]

33 **Freidja ML**, Tarhouni K, Toutain B, Fassot C, Loufrani L, Henrion D. The AGE-breaker ALT-711 restores high blood flow-dependent remodeling in mesenteric resistance arteries in a rat model of type 2 diabetes. *Diabetes* 2012; **61**: 1562-1572 [PMID: 22415880 DOI: 10.2337/db11-0750]

34 **Engelen L**, Stehouwer CD, Schalkwijk CG. Current therapeutic interventions in the glycation pathway: evidence from clinical studies. *Diabetes Obes Metab* 2013; **15**: 677-689 [PMID: 23279611 DOI: 10.1111/dom.12058]

35 **Thornalley PJ**. Use of aminoguanidine (Pimagedine) to prevent the formation of advanced glycation endproducts. *Arch Biochem Biophys* 2003; **419**: 31-40 [PMID: 14568006 DOI: 10.1016/j.abb.2003.08.013]

P- Reviewer: Gómez-Sáez J, Traub M **S- Editor**: Ji FF
L- Editor: A **E- Editor**: Liu SQ

World Journal of Diabetes

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4239/wjd.v6.i9.1113

World J Diabetes 2015 August 10; 6(9): 1113-1121
ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Prospective Study

Epigenetic profiles of pre-diabetes transitioning to type 2 diabetes and nephropathy

Thomas A VanderJagt, Monica H Neugebauer, Marilee Morgan, Donald W Bowden, Vallabh O Shah

Thomas A VanderJagt, Monica H Neugebauer, Vallabh O Shah, Department of Biochemistry and Molecular Biology, University of New Mexico School of Medicine, Albuquerque, NM 87131, United States

Marilee Morgan, MIND Institute, Albuquerque, NM 87106, United States

Donald W Bowden, Center for Diabetes Research, Wake Forest School of Medicine, Winston-Salem, NC 27157, United States

Author contributions: All authors contributed to this manuscript.

Supported by The grants from the National Center for Research Resources, No. 5P20RR016480-12; The National Institute of General Medical Sciences of the NIH, No. 8P20GM103451-12; the partial support from the National Center for Advancing Translational Sciences of the National Institutes of Health, No. 8UL1TR000041; the University of New Mexico Clinical and Translational Science Center; the cost for clinical phenotyping and payments to participants was supported under a UNM Health Sciences Center-based Cardiovascular and Metabolic Diseases Signature Program.

Institutional review board statement: The study protocol was approved by the Human Subject Research Review Committee of the University of New Mexico Health Sciences Center.

Clinical trial registration statement: The Clinical trial registration is not applicable for our study.

Informed consent statement: All participants rendered written informed consent.

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

Data sharing statement: We will coordinate data sharing and make available both the raw data and analyzed data upon request.

Correspondence to: Vallabh O Shah, PhD, FASN, Professor and Sr Fellow New Mexico Center for the Advancement of Research, Engagement, and Science on Health Disparities (NM CARES HD), Department of Biochemistry and Molecular Biology, University of New Mexico School of Medicine, 2211 Lomas Blvd, NE, Albuquerque, NM 87131, United States. vshah@salud.unm.edu
Telephone: +1-505-2729615
Fax: +1-505-2722614

Received: February 27, 2015
Peer-review started: February 27, 2015
First decision: May 14, 2015
Revised: June 19, 2015
Accepted: July 11, 2015
Article in press: July 14, 2015
Published online: August 10, 2015

Abstract

AIM: To examine DNA methylation profiles in a longitudinal comparison of pre-diabetes mellitus (Pre-DM) subjects who transitioned to type 2 diabetes mellitus (T2DM).

METHODS: We performed DNA methylation study in bisulphite converted DNA from Pre-DM (n = 11) at baseline and at their transition to T2DM using Illumina Infinium HumanMethylation27 BeadChip, that enables the query of 27578 individual cytosines at CpG loci throughout the genome, which are focused on the promoter regions of 14495 genes.

RESULTS: There were 694 CpG sites hypomethylated and 174 CpG sites hypermethylated in progression from

Pre-DM to T2DM, representing putative genes involved in glucose and fructose metabolism, inflammation, oxidative and mitochondrial stress, and fatty acid metabolism. These results suggest that this high throughput platform is able to identify hundreds of prospective CpG sites associated with diverse genes that may reflect differences in Pre-DM compared with T2DM. In addition, there were CpG hypomethylation changes associated with a number of genes that may be associated with development of complications of diabetes, such as nephropathy. These hypomethylation changes were observed in all of the subjects.

CONCLUSION: These data suggest that some epigenomic changes that may be involved in the progression of diabetes and/or the development of complications may be apparent at the Pre-DM state or during the transition to diabetes. Hypomethylation of a number of genes related to kidney function may be an early marker for developing diabetic nephropathy.

Key words: Epigenetic changes; Pre-diabetes; Diabetes; Nephropathy

Core tip: Many independent predictors of diabetes including markers of metabolic dysfunction (high body mass index, hypertension, low HDL and smoking) were significantly increased early on in pre-diabetes mellitus (Pre-DM) and sustained in diabetes groups. The innovation in high-throughput epigenome of DNA methylation studies suggests that some epigenomic changes that may be involved in the progression of diabetes and/or the development of complications may be apparent at the Pre-DM state or during the transition to diabetes.

VanderJagt TA, Neugebauer MH, Morgan M, Bowden DW, Shah VO. Epigenetic profiles of pre-diabetes transitioning to type 2 diabetes and nephropathy. *World J Diabetes* 2015; 6(9): 1113-1121 Available from: URL: http://www.wjgnet.com/1948-9358/full/v6/i9/1113.htm DOI: http://dx.doi.org/10.4239/wjd.v6.i9.1113

INTRODUCTION

Pre-diabetes mellitus (Pre-DM) is a condition characterized by elevated blood glucose concentrations that denote the incipient development of type 2 diabetes mellitus (T2DM), along with its co-morbid conditions of cardiovascular disease and renal disease. The most common definitions of Pre-DM refer to impaired glucose tolerance (IGT) and impaired fasting glucose (IFG). IGT and IFG are assumed to define categories of glycemia associated with an increased risk of developing diabetes[1-3]. It is estimated that roughly 86 million individuals in the United States aged 21 years and older have Pre-DM. People with Pre-DM are 5-15 times more likely to develop T2DM than are people with normal glucose values[1,4]. The risk of people with IGT and/or IFG developing T2DM is not uniform. An analysis of several prospective studies showed that the incidence rates of developing T2DM in people with IGT ranged from 35.8 to 87.3 per 1000 person-years[5]. Environmental exposures, sedentary lifestyle, and high calorie, high-fat diets correlate with the development of metabolic syndrome including obesity and insulin resistance. All of these factors influence the rate of progression of Pre-DM. Progression to T2DM among those with Pre-DM is not inevitable and also is variable in terms of development of complications related to T2DM. People with Pre-DM who lose weight and engage in moderate physical activity can prevent or delay T2DM and may even return their blood glucose levels to normal[6]. Similar to the prevalence of Pre-DM and T2DM in the United States, estimates suggest that more than 31 million people in the United States are affected by kidney disease, with less than 500000 (< 0.2%) having kidney failure treated by dialysis or transplantation. T2DM is an important independent risk factor for kidney disease in the United States and almost half of all new cases of End Stage Renal Disease (ESRD) are due to diabetic nephropathy. A tremendous amount of work has been done to better understand diabetic nephropathy and the risk of progression to ESRD and much of the work in renoprotection has focused on this population.

The etiological origins of T2DM are complex. A data-mining approach, which analyzed over 12 million Medline records to identify factors associated with the pathology of T2DM, identified epigenetic changes as among the most important causal factors in the pathogenesis of T2DM[7]. The epigenome is increasingly gaining acceptance as playing an important role in diabetes and obesity, and the role of both nutritional status and endocrine disruptors would appear to be major factors in these conditions[8-10]. Initial observations indicating a role for environmental cues in establishing epigenetic patterns came from studies of the agouti mouse model with offspring suffering from obesity, hyperinsulinaemia and diabetes[11]. In human studies it has been shown that trans-generational effects of nutrition may be passed on to future generations. In a study of historical records from Överkalix, Sweden, the grandsons of men who were well-nourished prior to puberty had an increased risk of developing T2DM[12].

A general defect in DNA methylation in T2DM is suggested by the observation that S-adenosylmethionine (SAM), the main physiologic donor of methyl groups, is decreased in erythrocytes of diabetic patients. In addition, decreased erythrocyte concentrations of SAM and other alterations were found to be associated with disease progression[13]. Methylation plays an important role in regulating gene expression, most likely including the expression of those genes essential for the strict maintenance of normal blood glucose levels. Expression

patterns that develop in response to changes in diet or in response to environmental factors are likely to become locked by DNA methylation early in development[14]. Methylation of DNA on specific cytosine residues in CpG islands, especially in promoter regions, leads to DNA hypermethylation, which generally is associated with lowering gene expression, while removal of methyl groups, leading to DNA hypomethylation, is generally associated with increasing gene expression. Methylation patterns have been suggested to be involved in the propagation of insulin resistance in insulin target tissues and, being a reversible modification, might also confer the adaptability of metabolism to loss of body weight.

In this longitudinal study, we used archived biological samples to examine the methylation patterns in DNA obtained from subjects at the time they were classified as Pre-DM and were also later obtained from the same subjects after they had transitioned to T2DM. All subjects in this cohort eventually developed diabetic nephropathy. This allowed for a longitudinal comparison of changes associated with transitioning from Pre-DM to T2DM. Our aims were two-fold: first, to obtain a global comparison of hyper- and hypomethylation patterns between the Pre-DM and T2DM states and analysis of these differences in terms of altered metabolic pathways; and second, to examine for methylation changes at the T2DM stage that might suggest early markers to future development of diabetic complications, specifically diabetic nephropathy.

MATERIALS AND METHODS

The study protocol was approved by the Human Subject Research Review Committee of the University of New Mexico Health Sciences Center. Genome-wide screening for DNA methylation was carried out with the Infinium 27K methylation array (Illumina Infinium® HumanMethylation27 BeadChip, Illumina, San Diego, CA). Quantitative measurements of DNA methylation were determined for 27578 CpG dinucleotide spanning 14495 genes. This methodology combines bisulfite conversion of genomic DNA and whole-genome amplification with direct, array-based capture and enzymatic scoring of the CpG loci. Allele-specific single-base extension of the oligos on the BeadChip, using the captured DNA as a template, incorporates detectable labels on the BeadChip and determines the methylation profile for the sample. One microgram of DNA was treated with sodium bisulfite using the Zymo EZ DNA Methylation Kit to convert un-methylated cytosines to uracil, while methylated cytosines remain unchanged. The DNA was purified and quantified in preparation for whole genome amplification, followed by fragmentation and ethanol precipitation. The DNA was re-suspended in hybridization buffer and applied to the bead chip array for an overnight incubation. Following hybridization, the arrays were washed to eliminate un-hybridized and non-specifically hybridized DNA. The samples then underwent single base extension and staining followed by more washing. The arrays were allowed to dry and then scanned using the Illumina iScan system. Analysis of the scanned results is achieved using Illumina's GenomeStudio software in conjunction with the GenomeStudio methylation module. GenomeStudio Software is a modular analysis tool for genotyping, gene expression, and methylation applications. The Methylation Module allows users to combine Infinium methylation assay data with mRNA data, enabling convergence of data across gene expression and epigenetic analyses.

To evaluate the genes identified by the Infinium methylation assay for groupings that may identify metabolic pathways, the data were analyzed with ingenuity pathway analysis (IPA) (Ingenuity Systems, Redwood City, CA). Differentially expressed transcripts satisfying the statistical conditions were exported to IPA. This software determines the top canonical pathways by using the ratio of the number of genes in a given pathway that meet cutoff criteria divided by the total number of genes that constitute that pathway. The significance of a pathway for the data set reflects the likelihood that the pathway is associated with the dataset by random chance. The methylation assay results were analyzed and scored for significance of hyper and hypomethylation compared to controls. Output (Beta) was used in computations creating a *P*-value from a Diff score; DiffScore = 10*sgn(Beta $_{\text{Condition}}$ - Beta $_{\text{Reference}}$)*log10p. Level of significance related to DiffScore are as follows; *P*-value of 0.05, DiffScore = ± 13; For a *P*-value of 0.01, DiffScore = ± 22; For a *P*-value of 0.001, DiffScore = ± 33.

This procedure was carried out using DNA isolated from 11 Pre-DM non-Hispanic white male subjects when they were diagnosed with Pre-DM and repeated after transitioning to T2DM, and from two reference subjects used to establish a baseline. The Pre-DM samples and the T2DM samples were normalized to this baseline in order to determine expression changes that occur over the extended time period between Pre-DM and T2DM. The cohort was limited to 11 non-Hispanic white males to minimize confounding variables of ethnicity and gender. Blood leukocyte samples were taken at each stage and phenotype for clinical parameters and anthropomorphic measurements. Every patient's transition from Pre-DM to T2DM involved a higher body mass index (BMI), weight gain as well as higher levels of blood glucose and HbA1c. Clinical standards set by the American Diabetes Association were used to classify the subjects: fasting plasma glucose levels of 99 mg/dL or below are considered normal; plasma glucose levels between 100 to 125 mg/dL indicate Pre-DM; and plasma glucose levels of 126 mg/dL and higher indicate T2DM. Subjects with a normal plasma glucose but elevated HbA1c (5.7%-6.0%) were also classified as Pre-DM.

RESULTS

The mean age at Pre-DM diagnosis was 40.27 ± 5.46,

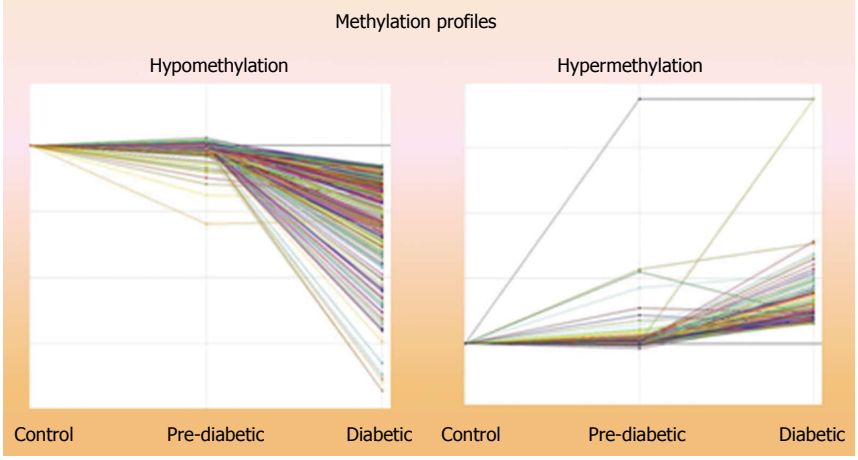

Figure 1 Global methylation profile depicting a total of 868 genes. Total of 694 were hypomethylated, 174 were hypermethylated.

Table 1 The top associated networks from ingenuity pathway analysis

Carbohydrate metabolism, small molecule biochemistry, cell signaling
Lipid metabolism, small molecule biochemistry, drug metabolism
Inflammatory response, cardiovascular system development and function, lymphoid tissue structure and development
Cellular function and maintenance, inflammatory response, cell-to-cell signaling and interaction
Cellular movement, hematological system development and function, immune cell trafficking

Table 2 The top biological functions identified by ingenuity pathway analysis and the number of genes involved

	# of genes
Diseases and disorders	
Inflammatory disease	218
Inflammatory response	202
Immunological disease	195
Respiratory disease	126
Hematological disease	112
Molecular and cellular functions	
Cellular growth and proliferation	230
Cell death	224
Cell-to-cell signaling and interaction	188
Cellular development	152
Physiological system development and function	
Hematological system development/function	214
Immune cell trafficking	137
Hematopoiesis	126
Tissue morphology	105
Cell-mediated immune response	85

the mean transition time was 7.09 ± 1.97 years, and the mean age at T2DM diagnosis was 47.36 ± 5.97 years of age. In the Pre-DM state the BMI = 32.2 ± 6.9, HbA1c = 5.5 ± 0.31, glucose = 99.1 ± 15.9 (mg/dL), and weight = 226.6 ± 68.9 (lbs). In the diabetic state, the BMI = 37.2 ± 6.9, HbA1c = 9.6 ± 2.15, glucose = 225.9 ± 78.8 (mg/dL), and weight = 261.4 ± 65.1 (lbs).

Global changes in methylation patterns

Comparisons of the epigenetic profiles of methylated CpG loci in DNA from 11 non-Hispanic white male revealed that 694 CpG sites were consistently hypo-methylated and 174 were hypermethylated in the DNA obtained at the time of transition to T2DM compared to the DNA obtained at Pre-DM (Figure 1). Analysis of the genes with IPA identified numerous putative genes associated with carbohydrate and lipid metabolism, inflammation, immune cell function and cell signaling, suggesting increased activities in these pathways at the T2DM state compared to the Pre-DM state. The five Top Associated Networks identified in the Ingenuity Pathway Analysis are summarized in Table 1.

The Top Biological Functions identified by IPA and the number of genes involved are summarized in Table 2. These include numerous genes that suggest changes that may be directly associated with or are early markers for diseases and disorders, molecular and cellular function and physiological system development and function, possibly related to the Pre-DM state transitioning to the T2DM state and related nephropathy.

Genes associated with kidney disease

The entire ensemble of genes was evaluated by literature search to identify key candidate genes involved in kidney disease. Sixteen genes were selected: *SLC22A12*, Transient Receptor Potential Melastatin subtype 6 (*TRPM6*), aquaporin 9 (*AQP9*), *HP*, *HPR*, *ABCC2*, alanine-glyoxylate aminotransferase (*AGXT*), *UGT2A3*, *HAL*, *HYAL2*, *SLC13A1*, *SERPINF1*, *CD22*, *SIGLEC5*, *NEU4*, and *NOX1*. These genes may be directly related to risk factors or biomarkers that signify kidney damage progression or vulnerability. Hypomethylation is seen in all sixteen of the genes. The methylation patterns are shown in Figure 2.

A total of 92 genes were hypomethylated and 54 were hypermethylated in all subjects. Analysis of the sub-group of sixteen genes (Figure 2) associated with kidney disease for genes which were hypomethylated in all subjects identified six genes. The data for these six genes are shown in Figure 3. These genes and their

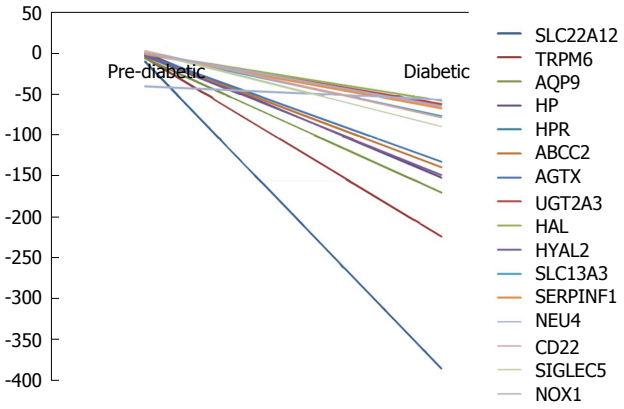

Figure 2 A total of 92 genes were hypomethylated and 54 were hypermethylated in all subjects using Illumina bead studio. Significantly hypomethylated genes were compared to putative genes identified in literature review forming of a sub-group of sixteen genes related to kidney disease. These sixteen genes are represented here as cumulative average DiffScore deviations from control comparisons. Scores of ± 13 *P*-value of 0.05, ± 22 *P*-value of 0.01.

associated products or functions are: *SLC22A12* (a urate transporter on the proximal tubule); *TRPM6* (a cation channel in the kidney); *AQP9* (an aquaporin); *HP* (haptoglobin, which binds plasma hemoglobin); *AGXT* (which is involved in oxalic acid secretion); *HYAL2* (a hyaluronidase).

DISCUSSION

This study demonstrated that there are a large number of methylation changes in the progression of Pre-DM to T2DM in a homogeneous longitudinal cohort of white males of which IPA of the associated genes identified numerous cellular pathways that potentially can be altered, leading to development and/or prediction of diabetes-related complications. Of particular interest, this study identified six genes that may be associated with/or predict the development of diabetic nephropathy. These six genes were hypomethylated in all subjects in the progression from Pre-DM to T2DM. The sample size was limited by the longitudinal observation of nearly a decade time period (approximately 7 years) required for the mean transition time from Pre-DM to T2DM. Although this sample size is small, the study identified a limited set of markers in this cohort that were hypomethylated in all subjects. Longitudinal studies similar to the present study but with larger numbers of subjects are especially difficult owing to the rare availability of DNA samples. However, the results from this study will aid in the design of future studies. For example, DNA from a range of subjects with diabetic nephropathy can be analyzed to confirm whether these changes in expression are observed in other ethnic groups during transition from Pre-DM to T2DM and also to compare with subjects who are resistant to developing T2DM related nephropathy.

Below is a brief discussion of the six genes

***SLC22A12*:** Uric acid, which is the metabolic end product of purine metabolism in humans, has protective antioxidant properties but can also be pro-oxidant. Urate, the ionized form of uric acid, scavenges potentially harmful radicals. Defective renal handling of urate is a frequent pathophysiologic factor in hyperuricemia. In response to genetic or environmental factors, such as diet, hyperuricemia may cause gout, nephrolithiasis, hypertension, and vascular disease. However, hypouricemia may also have pathological consequences. Humans have higher serum uric acid levels compared to other mammalian species; this is the result of genetic silencing of hepatic uricase, an enzyme that metabolizes uric acid into allantoin. Uric acid homeostasis is maintained by balance between production, intestinal secretion, and renal excretion. The kidney is important in the regulation of circulating uric acid levels through control of re-absorption of filtered urate and through uric acid excretion. In humans, urate transporters URAT1, MRP4, OAT1, and OAT3 play central roles in homeostasis. SLC22A12, a member of the organic anion transport family, encodes for the protein URAT1, which is a kidney-specific urate transporter that transports urate across the apical membrane of the proximal tubule various mutations in *SLC22A12* have been associated with renal disease. Given the importance of urate homeostasis, and the critical role of URAT1 activity in determining whether urate absorption *vs* secretion in balanced, epigenetic hypomethylation may be a determinant in the activity of URAT1[15-21] (Figure 3).

***TRPM6*:** TRPM6 is a member of the Transient Receptor Potential superfamily of cation channels, which are widely expressed and function in the regulation of absorption and secretion of cations. Many TRPs are expressed in kidney along the nephron. These channels are involved in hereditary as well as acquired kidney disorders. Increased expression of TRPM6 transporters is associated with diabetes mellitus and kidney damage in experimental animal models[22,23]. TRPM6 channels are primarily located in the renal distal convolution, the main site of active transcellular Ca(2+) and Mg(2+) transport in the kidney. The channels are regulated by many factors and hormones to maintain systemic concentrations of Ca(2+) and Mg(2+). Loss-of-function mutations in TRPM6 are a molecular cause of hypomagnesemia with secondary hypocalcemia. TRPM6 may be viewed as the gatekeeper of the body's Mg^{2+} balance although, even in the distal convolutions, multiple proteins involved in Mg^{2+} transport have been identified (TRPM6, proEGF, and FXYD2 which is the Na^+/K^+-ATPase gamma-subunit). Drug treatment, acid-base status, and several hormones have been shown to regulate TRPM6 expression[24-27].

***AQP9*:** AQP are integral membrane channels for the transfer of water, and in some cases, small solutes across the membrane. Aquaporins are conserved in bacteria, plants, and animals. There are more than 10

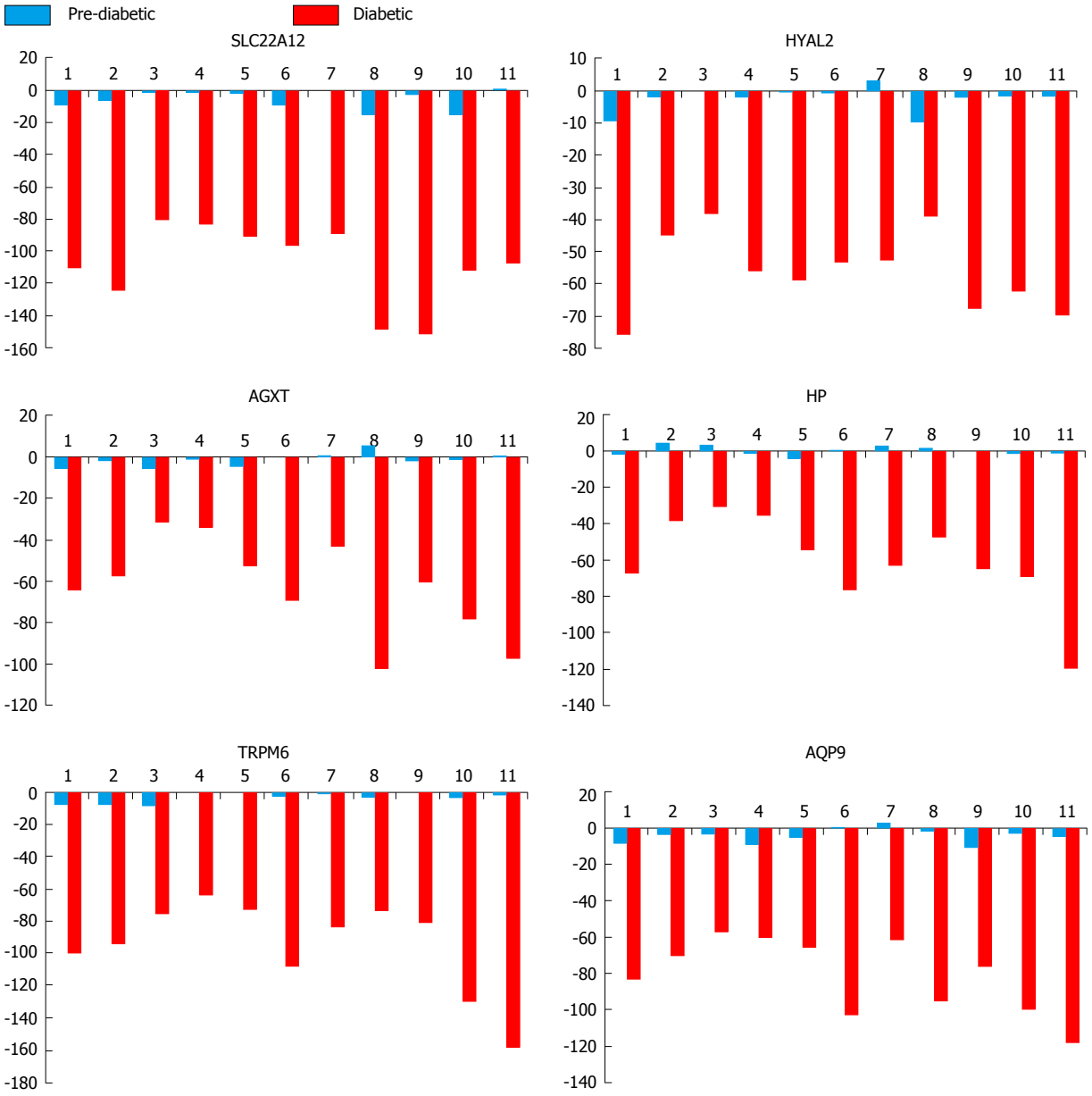

Figure 3 Hypo-methylated kidney disease associated gene loci. Six putative genes related to kidney disease were hypomethylated across patients (numbered 1-11) compared to controls (n = 2). Red bars indicate diabetic time point and blue bars representing prediabetic time point. Data represented as DiffScore with ± 13 equating to P-value 0.05, ± 22 equating to P-value of 0.01, and ± 33 equating to P-value of 0.001. SLC22A12 (a urate transporter on the proximal tubule); TRPM6 (a cation channel in the kidney); AQP9 (an aquaporin); HP (haptoglobin, which binds plasma hemoglobin); AGXT (alanine-glyoxylate aminotransferase which is involved in oxalic acid secretion); HYAL2 (a hyaluronidase).

isoforms of AQP. Several of the mammalian aquaporins (*e.g.*, AQP1, AQP2, AQP4, and AQP5) are selective for the passage of water; others also transport glycerol (*e.g.*, AQP3 and AQP8) and even larger solutes (AQP9). The human aquaporins, AQP3, AQP7, AQP8 and AQP9 are also permeable to ammonia. AQP9 is an aquaporin which stimulates urea transport and allows passage of a wide variety of non-charged solutes. AQP9 is expressed in numerous tissues and is especially abundant in liver. AQP9 as well as other AQPs also are expressed in kidney. The ammonia-transporting AQPs, including AQP9, supplement the ammonia transport of the Rhesus proteins; AQP9 also supplements the urea transporters. AQP9 can also transport arsenic trioxide[28-32]. Given the wide distribution of the AQPs, including AQP9, in is unclear whether epigenetic hypomethylation of APQ9 in diabetes would contribute to development of diabetic nephropathy. One possibility is that the special properties of AQP9 in urea transport may require highly controlled expression in tissues, such as kidney, which have important roles in urea transport.

HP: The *HP* gene encodes for haptoglobin (Hp) which functions to bind free plasma hemoglobin, thereby helping to prevent loss of iron through the kidney and protecting the kidneys from damage by hemoglobin. Iron status is influenced by environmental and genetic factors. The genetic polymorphism of Hp has been

shown to affect iron turnover. Hp captures hemoglobin in plasma to allow hepatic recycling of heme iron, which helps to prevent kidney damage during hemolysis. Hp acts as an anti-oxidant by binding hemoglobin. Two common alleles for Hp (1 and 2) produce three common Hp genotypes: Hp1-1, Hp2-1, and Hp2-2. The protein encoded by Hp1-1 provides superior antioxidant protection compared with that encoded by Hp2-2. Hp genotype is an independent risk factor for complications; individuals with Hp2-2 are more likely to develop nephropathy, retinopathy, and cardiovascular disease as compared with those with Hp2-1 or Hp1-1. In diabetic patients, urinary Hp levels and genotype predict renal functional decline. Aged animals are especially sensitive to the nephrotoxicity of hemoglobin. Hp synthesis is primarily a function of liver where Hp up regulation is a major stress response. However, in acute kidney injury, Hp synthesis in the proximal tubules is a major stress response[33-39].

***AGXT*:** The *AGXT* gene codes for the peroxisomal enzyme AGXT, which converts glyoxylate into glycine using L-alanine as the amino-group donor. Mutations in the AGXT are responsible for primary hyperoxaluria type 1 (PH1), which is a rare disease characterized by excessive hepatic oxalate production. When AGXT activity is absent, glyoxylate is converted to oxalate. Oxalate forms insoluble calcium salts that accumulate in the kidney. PH1 patients are at risk for recurrent deposition of calcium oxalate in the renal pelvis/urinary tract, deposition of calcium oxalate in the renal parenchyma, or ESRD. The PH1 is mostly due to single point mutations on the *AGXT* gene; more than 150 so far been identified[40-42]. The epigenetic hypomethylation of AGXT (Figure 3), where hypomethylation generally is associated with enhanced expression, would seem counter to a role for AGXT where PH1 is associated with diminished activity. However, in a recent cluster analysis of microarray expression data for genes associated with T2DM and nephropathy, AGXT was identified as one of the more highly expressed genes[43].

***HYAL2*:** Hyaluronidases degrade hyaluronan, one of the major glycosaminoglcans of the extracellular matrix. The human genome contains six hyaluronidase-like genes. HYAL2 and HYAL1 are the major mammalian hyaluronidases in somatic tissues. They work together to degrade high molecular weight hyaluronan to tetrasaccharides. Initially large hyaluronan fragments (20 kD) are generated at the cell surface from digestion by the glycosylphosphatidyl-inositol-anchored HYAL2. These fragments are internalized and further digested by HYAL1. Alterations in hyaluronan have been reported in numerous renal diseases. The accumulation of hyaluronan in the renal cortex is observed in inflammatory renal diseases. In addition, the large fragments of hyaluronan produced by HYAL2 display inflammatory effects *in vitro* and may contribute to immune renal injury. Increased activity of renal hyaluronidase occurs in streptozotocin-induced diabetic rats; this activity increases in multiple areas of the kidney during the progression of diabetic nephropathy[44-47].

There are a number of limitations to this study. The sample size was small, reflecting the challenges in obtaining DNA samples from subjects at the Pre-DM and T2DM stages of patients who eventually developed diabetic nephropathy. The six selected kidney disease-associated gene based on literature evaluation, which were hypomethylated in all of the subjects, suggests but does not prove that the expression levels of these genes were up regulated during the progression to T2DM. In addition, the hypomethylation of these genes does not predict the interval of time before the development of nephropathy. Nevertheless, the fact that all of the subjects exhibited hypomethylation of these genes raises the question whether these changes might be predictive of diabetic nephropathy.

COMMENTS

Background

Type 2 diabetes mellitus (T2DM) affects more than 29 million in United States and about 79 million adults have pre-diabetes mellitus (Pre-DM). Environmental exposures, sedentary lifestyle, and high calorie, high-fat diets correlate with the development of metabolic syndrome including obesity and insulin resistance. All of these factors influence the rate of progression of Pre-DM. Recent studies suggest that gene-environment interactions relevant for T2DM are at least partly regulated by epigenomic mechanisms.

Research frontiers

The epigenome is increasingly gaining acceptance as playing an important role in diabetes and obesity, and the role of both nutritional status and endocrine disruptors would appear to be major factors in these conditions. A general defect in DNA methylation in T2DM is suggested by the observation that S-adenosylmethionine (SAM), the main physiologic donor of methyl groups, is decreased in erythrocytes of diabetic patients. In addition, decreased erythrocyte concentrations of SAM and other alterations were found to be associated with disease progression.

Innovations and breakthroughs

The study demonstrated that there are a large number of methylation changes in the progression of Pre-DM to T2DM. The study results revealed that 694 CpG sites were consistently hypomethylated and 174 were hypermethylated in the DNA obtained at the time of transition to T2DM compared to the DNA obtained at Pre-DM. The putative genes identified are associated with carbohydrate and lipid metabolism, inflammation, immune cell function and cell signaling, suggesting increased activities in these pathways at the T2DM state compared to the Pre-DM state. The authors further observed methylation changes in six candidate genes in all patients at the T2DM stage with nephropathy suggesting future development of diabetic complications.

Applications

Characterizing the epigenomic components that may regulate the transcriptional potential of a cell and contribute to the etiology, severity and progression of Pre-DM to T2DM and to complications including kidney disease will provide novel insights into disease pathogenesis and therapeutic approaches. This knowledge will enhance our ability to investigate, diagnose and ameliorate T2DM and kidney disease with a significant epigenomic component.

Peer-review

It is an interesting prospective study, analyzing DNA methylation profiling in

11 pre diabetic and 2 control individuals. In addition hypomethylation may be associated to difference genes in the nephropaty progression.

REFERENCES

1 **Center for Disease Control and Prevention**. National Diabetes Fact Sheet. 2011. Available from: URL: http://www.cdc.gov/diabetes/pubs/factsheet11.htm
2 **International Diabetes Federation**. IDF Diabetes Atlas. 6th ed. Available from: URL: http://www.idf.org/diabetesatlas/
3 **Nathan DM**, Davidson MB, DeFronzo RA, Heine RJ, Henry RR, Pratley R, Zinman B. Impaired fasting glucose and impaired glucose tolerance: implications for care. *Diabetes Care* 2007; **30**: 753-759 [PMID: 17327355 DOI: 10.2337/dc07-9920]
4 **DeFronzo RA**, Abdul-Ghani M. Assessment and treatment of cardiovascular risk in prediabetes: impaired glucose tolerance and impaired fasting glucose. *Am J Cardiol* 2011; **108**: 3B-24B [PMID: 21802577 DOI: 10.1016/j.amjcard.2011.03.013]
5 **Edelstein SL**, Knowler WC, Bain RP, Andres R, Barrett-Connor EL, Dowse GK, Haffner SM, Pettitt DJ, Sorkin JD, Muller DC, Collins VR, Hamman RF. Predictors of progression from impaired glucose tolerance to NIDDM: an analysis of six prospective studies. *Diabetes* 1997; **46**: 701-710 [PMID: 9075814 DOI: 10.2337/diab.46.4.701]
6 **Colberg SR**, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, Chasan-Taber L, Albright AL, Braun B. Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement. *Diabetes Care* 2010; **33**: e147-e167 [PMID: 21115758 DOI: 10.2337/dc10-9990]
7 **Wren JD**, Garner HR. Data-mining analysis suggests an epigenetic pathogenesis for type 2 diabetes. *J Biomed Biotechnol* 2005; **2005**: 104-112 [PMID: 16046815 DOI: 10.1155/JBB.2005.104]
8 **Gallou-Kabani C**, Junien C. Nutritional epigenomics of metabolic syndrome: new perspective against the epidemic. *Diabetes* 2005; **54**: 1899-1906 [PMID: 15983188 DOI: 10.2337/diabetes.54.7.1899]
9 **Gallou-Kabani C**, Vigé A, Junien C. Lifelong circadian and epigenetic drifts in metabolic syndrome. *Epigenetics* 2007; **2**: 137-146 [PMID: 17965598 DOI: 10.4161/epi.2.3.4897]
10 **Jirtle RL**, Skinner MK. Environmental epigenomics and disease susceptibility. *Nat Rev Genet* 2007; **8**: 253-262 [PMID: 17363974 DOI: 10.1038/nrg2045]
11 **Miltenberger RJ**, Mynatt RL, Wilkinson JE, Woychik RP. The role of the agouti gene in the yellow obese syndrome. *J Nutr* 1997; **127**: 1902S-1907S [PMID: 9278579]
12 **Kaati G**, Bygren LO, Edvinsson S. Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. *Eur J Hum Genet* 2002; **10**: 682-688 [PMID: 12404098 DOI: 10.1038/sj.ejhg.5200859]
13 **Poirier LA**, Brown AT, Fink LM, Wise CK, Randolph CJ, Delongchamp RR, Fonseca VA. Blood S-adenosylmethionine concentrations and lymphocyte methylenetetrahydrofolate reductase activity in diabetes mellitus and diabetic nephropathy. *Metabolism* 2001; **50**: 1014-1018 [PMID: 11555831 DOI: 10.1053/meta.2001.25655]
14 **Gluckman PD**, Lillycrop KA, Vickers MH, Pleasants AB, Phillips ES, Beedle AS, Burdge GC, Hanson MA. Metabolic plasticity during mammalian development is directionally dependent on early nutritional status. *Proc Natl Acad Sci USA* 2007; **104**: 12796-12800 [PMID: 17646663 DOI: 10.1073/pnas.0705667104]
15 **Hediger MA**, Johnson RJ, Miyazaki H, Endou H. Molecular physiology of urate transport. *Physiology* (Bethesda) 2005; **20**: 125-133 [PMID: 15772301 DOI: 10.1152/physiol.00039.2004]
16 **Sakurai H**. Urate transporters in the genomic era. *Curr Opin Nephrol Hypertens* 2013; **22**: 545-550 [PMID: 23852331 DOI: 10.1097/MNH.0b013e328363ffc8]
17 **Bobulescu IA**, Moe OW. Renal transport of uric acid: evolving concepts and uncertainties. *Adv Chronic Kidney Dis* 2012; **19**: 358-371 [PMID: 23089270 DOI: 10.1053/j.ackd.2012.07.009]
18 **Anzai N**, Jutabha P, Amonpatumrat-Takahashi S, Sakurai H. Recent advances in renal urate transport: characterization of candidate transporters indicated by genome-wide association studies. *Clin Exp Nephrol* 2012; **16**: 89-95 [PMID: 22038265 DOI: 10.1007/s10157-011-0532-z]
19 **So A**, Thorens B. Uric acid transport and disease. *J Clin Invest* 2010; **120**: 1791-1799 [PMID: 20516647 DOI: 10.1172/JCI42344]
20 **Tasic V**, Hynes AM, Kitamura K, Cheong HI, Lozanovski VJ, Gucev Z, Jutabha P, Anzai N, Sayer JA. Clinical and functional characterization of URAT1 variants. *PLoS One* 2011; **6**: e28641 [PMID: 22194875 DOI: 10.1371/journal.pone.0028641]
21 **Enomoto A**, Endou H. Roles of organic anion transporters (OATs) and a urate transporter (URAT1) in the pathophysiology of human disease. *Clin Exp Nephrol* 2005; **9**: 195-205 [PMID: 16189627 DOI: 10.1007/s10157-005-0368-5]
22 **Lee CT**, Lien YH, Lai LW, Chen JB, Lin CR, Chen HC. Increased renal calcium and magnesium transporter abundance in streptozotocin-induced diabetes mellitus. *Kidney Int* 2006; **69**: 1786-1791 [PMID: 16557223 DOI: 10.1038/sj.ki.5000344]
23 **Menè P**, Punzo G, Pirozzi N. TRP channels as therapeutic targets in kidney disease and hypertension. *Curr Top Med Chem* 2013; **13**: 386-397 [PMID: 23432067 DOI: 10.2174/1568026611313030013]
24 **Dietrich A**, Chubanov V, Gudermann T. Renal TRPathies. *J Am Soc Nephrol* 2010; **21**: 736-744 [PMID: 20395377 DOI: 10.1681/ASN.2009090948]
25 **Dimke H**, Hoenderop JG, Bindels RJ. Hereditary tubular transport disorders: implications for renal handling of Ca2+ and Mg2+. *Clin Sci* (Lond) 2010; **118**: 1-18 [PMID: 19780717 DOI: 10.1042/CS20090086]
26 **Woudenberg-Vrenken TE**, Bindels RJ, Hoenderop JG. The role of transient receptor potential channels in kidney disease. *Nat Rev Nephrol* 2009; **5**: 441-449 [PMID: 19546862 DOI: 10.1038/nrneph.2009.100]
27 **Hsu YJ**, Hoenderop JG, Bindels RJ. TRP channels in kidney disease. *Biochim Biophys Acta* 2007; **1772**: 928-936 [PMID: 17346947 DOI: 10.1016/j.bbadis.2007.02.001]
28 **Litman T**, Søgaard R, Zeuthen T. Ammonia and urea permeability of mammalian aquaporins. *Handb Exp Pharmacol* 2009; (**190**): 327-358 [PMID: 19096786 DOI: 10.1007/978-3-540-79885-9_17]
29 **Verkman AS**, Mitra AK. Structure and function of aquaporin water channels. *Am J Physiol Renal Physiol* 2000; **278**: F13-F28 [PMID: 10644652]
30 **Takata K**, Matsuzaki T, Tajika Y. Aquaporins: water channel proteins of the cell membrane. *Prog Histochem Cytochem* 2004; **39**: 1-83 [PMID: 15242101 DOI: 10.1016/j.proghi.2004.03.001]
31 **Sugiura K**, Aste N, Fujii M, Shimada K, Saito N. Effect of hyperosmotic stimulation on aquaporins gene expression in chick kidney. *Comp Biochem Physiol A Mol Integr Physiol* 2008; **151**: 173-179 [PMID: 18621138 DOI: 10.1016/j.cbpa.2008.06.014]
32 **Liu Z**. Roles of vertebrate aquaglyceroporins in arsenic transport and detoxification. *Adv Exp Med Biol* 2010; **679**: 71-81 [PMID: 20666225 DOI: 10.1007/978-1-4419-6315-4_6]
33 **Delanghe JR**, Langlois MR. Haptoglobin polymorphism and body iron stores. *Clin Chem Lab Med* 2002; **40**: 212-216 [PMID: 12005209 DOI: 10.1515/CCLM.2002.035]
34 **Nakhoul FM**, Miller-Lotan R, Awaad H, Asleh R, Levy AP. Hypothesis--haptoglobin genotype and diabetic nephropathy. *Nat Clin Pract Nephrol* 2007; **3**: 339-344 [PMID: 17525716 DOI: 10.1038/ncpneph0467]
35 **Asleh R**, Levy AP. In vivo and in vitro studies establishing haptoglobin as a major susceptibility gene for diabetic vascular disease. *Vasc Health Risk Manag* 2005; **1**: 19-28 [PMID: 17319095 DOI: 10.2147/vhrm.1.1.19.58930]
36 **Orchard TJ**, Sun W, Cleary PA, Genuth SM, Lachin JM, McGee P, Paterson AD, Raskin P, Anbinder Y, Levy AP. Haptoglobin genotype and the rate of renal function decline in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. *Diabetes* 2013; **62**: 3218-3223 [PMID: 23761102 DOI: 10.2337/db13-0256]
37 **Bhensdadia NM**, Hunt KJ, Lopes-Virella MF, Michael Tucker J, Mataria MR, Alge JL, Neely BA, Janech MG, Arthur JM. Urine haptoglobin levels predict early renal functional decline in patients with type 2 diabetes. *Kidney Int* 2013; **83**: 1136-1143 [PMID: 23536133 DOI: 10.1038/ki.2013.57]
38 **Nath KA**, Grande JP, Farrugia G, Croatt AJ, Belcher JD, Hebbel

RP, Vercellotti GM, Katusic ZS. Age sensitizes the kidney to heme protein-induced acute kidney injury. *Am J Physiol Renal Physiol* 2013; **304**: F317-F325 [PMID: 23195679 DOI: 10.1152/ajprenal.00606.2012]

39 **Zager RA**, Vijayan A, Johnson AC. Proximal tubule haptoglobin gene activation is an integral component of the acute kidney injury "stress response". *Am J Physiol Renal Physiol* 2012; **303**: F139-F148 [PMID: 22573378 DOI: 10.1152/ajprenal.00168.2012]

40 **Hernández-Fernaud JR**, Salido E. Differential expression of liver and kidney proteins in a mouse model for primary hyperoxaluria type I. *FEBS J* 2010; **277**: 4766-4774 [PMID: 20977670 DOI: 10.1111/j.1742-4658.2010.07882.x]

41 **Cellini B**, Oppici E, Paiardini A, Montioli R. Molecular insights into primary hyperoxaluria type 1 pathogenesis. *Front Biosci* (Landmark Ed) 2012; **17**: 621-634 [PMID: 22201765 DOI: 10.2741/3948]

42 **Danpure CJ**. Molecular aetiology of primary hyperoxaluria type 1. *Nephron Exp Nephrol* 2004; **98**: e39-e44 [PMID: 15499210 DOI: 10.1159/000080254]

43 **Guttula SV**, Rao AA, Sridhar GR, Chakravarthy MS, Nageshwararo K, Rao PV. Cluster analysis and phylogenetic relationship in biomarker identification of type 2 diabetes and nephropathy. *Int J Diabetes Dev Ctries* 2010; **30**: 52-56 [PMID: 20431808 DOI: 10.4103/0973-3930.60003]

44 **Sun L**, Feusi E, Sibalic A, Beck-Schimmer B, Wüthrich RP. Expression profile of hyaluronidase mRNA transcripts in the kidney and in renal cells. *Kidney Blood Press Res* 1998; **21**: 413-418 [PMID: 9933825 DOI: 10.1159/000025893]

45 **Declèves AE**, Caron N, Voisin V, Legrand A, Bouby N, Kultti A, Tammi MI, Flamion B. Synthesis and fragmentation of hyaluronan in renal ischaemia. *Nephrol Dial Transplant* 2012; **27**: 3771-3781 [PMID: 22529164 DOI: 10.1093/ndt/gfs098]

46 **Andre B**, Duterme C, Van Moer K, Mertens-Strijthagen J, Jadot M, Flamion B. Hyal2 is a glycosylphosphatidylinositol-anchored, lipid raft-associated hyaluronidase. *Biochem Biophys Res Commun* 2011; **411**: 175-179 [PMID: 21740893 DOI: 10.1016/j.bbrc.2011.06.125]

47 **Ikegami-Kawai M**, Suzuki A, Karita I, Takahashi T. Increased hyaluronidase activity in the kidney of streptozotocin-induced diabetic rats. *J Biochem* 2003; **134**: 875-880 [PMID: 14769877 DOI: 10.1093/jb/mvg214]

P- Reviewer: Hwu CM, Sanlioglu AD, Sanchez R, Wu CS
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Liu SQ

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