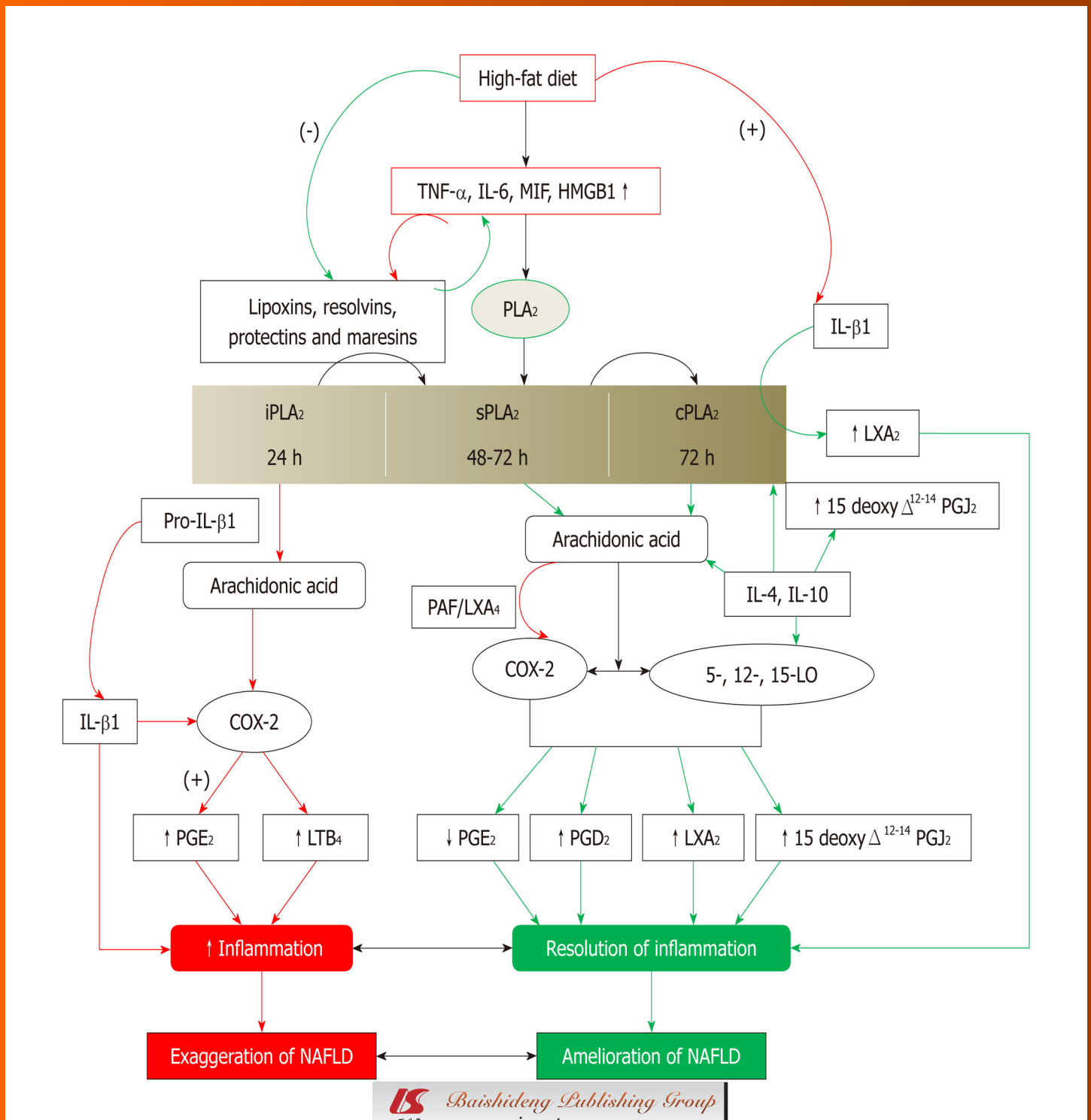
ISSN 1948-9358 (online)

World Journal of *Diabetes*

World J Diabetes 2011 November 15; 2(11): 176-210



Baishideng Publishing Group
www.wjgnet.com

World Journal of Diabetes

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

Editorial Board

2010-2015

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

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

Denmark

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

Egypt

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

Finland

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

France

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

Germany

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

Greece

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

Hungary

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

India

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

Ireland

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

Iran

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

Israel

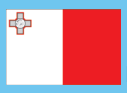
Shimon Efrat, *Tel Aviv*
Oren Froy, *Rehovot*
Eleazar Shafrir, *Jeusalem*
Haim Werner, *Tel Aviv*
Marina S Zimlichman, *Holon*

Italy

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

Japan

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



Malta

Charles Savona Ventura, *Msida*

Netherlands

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

New Zealand

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

Oman

Jumana S Saleh, *Muscat*

Poland

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

Romania

Elena Ganea, *Bucharest*

Singapore

S Thameem Dheen, *Singapor*
Yung Seng Lee, *Singapore*

South Korea

Won Mi Hwang, *Seoul*
Eui-Bae Jeung, *Chungbuk*
Ju-Hee Kang, *Incheon*
Sin Gon Kim, *Seongbuk-Gu*
Young-Gyu Ko, *Seoul*
Kang-Beom Kwon, *Chonbuk*
Byung-Hyun Park, *Jeonbuk*
Seungjoon Park, *Seoul*
Kun-Ho Yoon, *Seocho-Gu*

Spain

M Lusia Bonet, *Palma de Mallorca*
Manuel VCarrera, *Barcelona*
Justo P Castaño, *Cordoba*
Javier Espino, *Badajoz*
Oreste Gualillo, *Santiago*
Emilio Herrera, *Madrid*
Amelia Marti, *Pamplona*
Ricardo V García Mayor, *Vigo*
JF Navarro-González, *Tenerife*
Maria Javier Ramirez, *Pamplona*
José MG Sáez, *Barcelona*
Helmut Schröder, *Barcelona*
Segundo Carmen Segundo, *Cádiz*
SimRafael Simó, *Barcelona*

Sweden

Mozhgan Dorkhan, *Malmö*
Shao-Nian Yang, *Stockholm*
Weili Xu, *Stockholm*

Switzerland

Pascal Bovet, *Lausanne*

Thailand

N Charoenphandhu, *Bangkok*
Viroj Wiwanitkit, *Bangkok*

Turkey

Ugur Cavlak, *Denizli*
Teoman Dogru, *Ankara*
Abdurrahman F Fidan, *Afyonkarahisar*
Muammer Karadeniz, *Bornova-Izmir*
Cevdet Kaya, *Istanbul*
Fahrettin Kelestimur, *Kayseri*
Mustafa Şahin, *Mecburi Hizmet*
Ilker Tasci, *Ankara*
Belma Turan, *Ankara*

United Arab Emirates

Ernest A Adeghate, *Al Ain*
Samir M Awadallah, *Sharjah*

United Kingdom

Chen Bing, *Liverpool*
Peter John Grant, *Leeds*
Lora Katherine Heisler, *Cambridge*
Nigel Hoggard, *Scotland*
Andreas F Kolb, *Scotland*
Stefan Marciniak, *Cambridge*
Moffat Joha Nyirenda, *Scotland*
Thozhukat Sathyapalan, *Yorkshire*
Latika Sibal, *Newcastle upon Tyne*
Abd A Tahrani, *Birmingham*
G Neil Thomas, *Birmingham*

United States

Hwyda A Arafat, *Pennsylvania*
Sanford A Asher, *Pennsylvania*
Daniel C Batlle, *Illinois*
David SH Bell, *Alabama*
Donald W Bowden, *North Carolina*
Lu Cai, *Kentucky*
Jack D Caldwell, *Pennsylvania*
Anna C Calkin, *California*
Roberto A Calle, *Connecticut*
Heping Cao, *Los Angeles*
Krista Casazza, *Birmingham*
Xiao-Li Chen, *Saint Paul*
Craig Ian Coleman, *Connecticut*
Patricia Ann D'Amore, *Massachusetts*
Michael Harvey Davidson, *Illinois*
Samuel C Durso, *Maryland*
Alexander M Efanov, *Indiana*
Amy Zhihong Fan, *Georgia*
Alessia Fornoni, *Florida*
Gunjan Y Gandhi, *Florida*
Raimund Hirschberg, *California*
Michael Francis Holick, *Massachusetts*
Rachel Mary Hudacko, *New Brunswick*
Hieronim Jakubowski, *New Jersey*
Marilyn Jefferson, *New York*
Hong-Lin Jiang, *Virginia*
Richard Evers Katholi, *Springfield*
Tomoshige Kino, *Bethesda*
Julienne K Kirk, *North Carolina*
Renu A Kowluru, *Michigan*
Lewis H Kuller, *Pennsylvania*
Blandine Laferrère, *New York*
Sang Yeoup Lee, *Mayo Clinic*
Cong-Jun Li, *Maryland*
Shuo Lin, Los *Angeles*
Dong-Min Liu, *Virginia*
Zhen-Qi Liu, *Charlottesville*
Jian-Xing Ma, *Oklahoma City*
Xin-Laing Ma, *Pennsylvania*
Kenneth Maiese, *Michigan*
Sridhar Mani, *Bronx*
Suresh Mathews, *Auburn*
Lauraar McCabe, *East Lansing*
Murielle Mimeault, *Nebraska*
Reema Mody, *Grayslake*
Mohammad R Movahed, *Tucson*
Charles B Nemeroff, *Georgia*
Steven Nissen, *Ohio*
Wei-Hong Pan, *Baton Rouge*
Inga Peter, *New York*
Gretchen A Piatt, *Pennsylvania*
Wei Qiao Qiu, *Massachusetts*
Cristina Rabadán-Diehl, *Maryland*
Rajendra S Raghow, *Memphis*
Swapnil Rajpathak , *New York*
Mohammed S Razzaque, *Boston*
Beverly AS Reyes, *Pennsylvania*
Juan M Saavedra, *Maryland*
Vallabh O Shah, *Albuquerque*
Carol Ann Shively, *North Carolina*
Anders AF Sima, *Michigan*
Rajan Singh, *Los Angeles*
Rakesh K Srivastava, *Texas*
Bangyan Stiles, *California*
Yu-Xiang Sun, *Houston*
Ya-Xiong Tao, *Alabama*
John A Tayek, *Torrance,*
John Gaylord Teeter, *Connecticut*
Carlos M Telleria, *South Dakota*
Michael L Traub, *Staten Island*
Guillermo E Umpierrez, *Georgia*
Margrit Urbanek, *Illinois*
Hong-Jun Wang, *Boston*
Mark E Williams, *Massachusetts*
Guangyu Wu, *Los Angeles*
Zhong-Jian Xie, *San Francisoco*
Yisang Yoon, *New York*
Yi-Hao Yu, *New York*
Kevin CJ Yuen, *Portland*
Cui-Lin Zhang, *Maryland*

Venezuela

Fuad Lechin, *Caracas*

WJD World Journal of Diabetes

Contents

Monthly Volume 2 Number 11 November 15, 2011

World Journal of Diabetes
Volume 2 Number 11 November 15, 2011

Contents

NAME OF JOURNAL
World Journal of Diabetes

LAUNCH DATE
April 15, 2010

SPONSOR
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

EDITING
Editorial Board of *World Journal of Diabetes,*
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: wjd@wjgnet.com
http://www.wjgnet.com

PUBLISHING
Baishideng Publishing Group Co., Limited,
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai,
Hong Kong, China
Fax: +852-3115-8812
Telephone: +852-5804-2046
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

SUBSCRIPTION
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
November 15, 2011

ISSN
ISSN 1948-9358 (online)

World Journal of Diabetes
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: wjd@wjgnet.com
http://www.wjgnet.com

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1948-9358/g_info_20100107165233.htm.

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

World Journal of Diabetes

Online Submissions: http://www.wjgnet.com/1948-9358office
wjd@wjgnet.com
doi:10.4239/wjd.v2.i11.176

World J Diabetes 2011 November 15; 2(11): 176-188
ISSN 1948-9358 (online)

EDITORIAL

A defect in the activities of Δ^6 and Δ^5 desaturases and pro-resolution bioactive lipids in the pathobiology of non-alcoholic fatty liver disease

Undurti N Das

Undurti N Das, UND Life Sciences, 13800 Fairhill Road, 321, Shaker Heights, OH 44120, United States
Undurti N Das, School of Biotechnology, Jawaharlal Nehru Technological University, Kakinada-533 003, India
Undurti N Das, Bio-Science Research Centre, Gayatri Vidya Parishad College of Engineering Visakhapatnam-530 048, India
Author contributions: Das UN solely contributed to this paper.
Supported by Ramalingaswami Fellowship of the Department of Biotechnology, India; a grant from the Defense Research and Development Organisation, New Delhi, India
Correspondence to: Undurti N Das, MD, FAMS, UND Life Sciences, 13800 Fairhill Road, 321, Shaker Heights, OH 44120, United States. undurti@hotmail.com
Telephone: +1-216-2315548 Fax: +1-928-8330316
Received: July 4, 2011 Revised: September 28, 2011
Accepted: October 31, 2011
Published online: November 15, 2011

Abstract

Non-alcoholic fatty liver disease (NAFLD) is a low-grade systemic inflammatory condition, since liver and adipose tissue tumor necrosis factor-α (TNF-α) and TNF receptor 1 transcripts and serum TNF-α levels are increased and IL-6$^{-/-}$ mice are less prone to NAFLD. Fatty liver damage caused by high-fat diets is associated with the generation of pro-inflammatory prostaglandin E_2 (PGE_2). A decrease in the levels of arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and the usefulness of EPA and DHA both in the prevention and management of NAFLD has been reported. AA, EPA and DHA and their anti-inflammatory products lipoxins (LXs), resolvins and protectins suppress IL-6 and TNF-α and PGE_2 production. These results suggest that the activities of Δ^6 and Δ^5 desaturases are reduced in NAFLD and hence, the dietary essential fatty acids, linoleic acid (LA) and α-linolenic acid (ALA) are not metabolized to their long-chain products AA, EPA and DHA, the precursors of anti-inflammatory molecules, LXs, resolvins and protectins that could prevent NAFLD. This suggests that an imbalance between pro- and anti-inflammatory bioactive lipids contribute to NAFLD. Hence, it is proposed that plasma and tissue levels of AA, EPA, DHA and LXs, resolvins and protectins could be used as predictors and prognostic biomarkers of NAFLD. It is suggested that the synthesis and use of more stable analogues of LXs, resolvins and protectins need to be explored in the prevention and management of NAFLD.

Key words: Prostaglandins; Lipids; Arachidonic acid; Eicosapentaenoic acid; Non-alcoholic fatty liver disease; Docosahexaenoic acid; Lipoxins; Resolvins; Protectins; Cytokines; Free radicals; Hyperlipidemia

Peer reviewer: Christa Buechler, Dr., Department of Internal Medicine I, Regensburg University Hospital, Franz Josef Strauss Allee 11, Regensburg 93042, Germany

Das UN. A defect in the activities of Δ^6 and Δ^5 desaturases and pro-resolution bioactive lipids in the pathobiology of non-alcoholic fatty liver disease. *World J Diabetes* 2011; 2(11): 176-188 Available from: URL: http://www.wjgnet.com/1948-9358/full/v2/i11/176.htm DOI: http://dx.doi.org/10.4239/wjd.v2.i11.176

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) appears to be an integral part of the metabolic syndrome that comprises a cluster of abnormalities (dysglycemia, dyslipidemia, hypertension, procoagulant tendency, *etc.*) with insulin resistance as a central pathogenic factor[1,2]. NAFLD is significantly associated with insulin resistance[3,4]. Subjects with NAFLD had significantly higher values of body mass index (BMI), waist circumference, hip circumference, fasting blood glucose, fasting insulin, total choles-

terol and serum triglycerides[5,6]. A reduction in hepatic insulin sensitivity due to triglyceride accumulation in liver has been documented[5]. Triglyceride storage in the liver could be a protective mechanism and does not necessarily impair insulin sensitivity nor contribute to liver damage. Current understanding suggests that inappropriate triglyceride storage and formation of harmful lipid derivatives or increased free fatty acids may be harmful. Despite much research, the exact pathophysiological mechanisms of NAFLD are not clear.

NAFLD AS A LOW-GRADE SYSTEMIC INFLAMMATORY CONDITION

NAFLD could be a low-grade systemic inflammatory condition since liver and adipose tissue tumor necrosis factor-α (TNF-α) and TNF receptor 1 transcripts[7] as well as serum TNF-α levels[8] are increased in patients with NAFLD and IL-6-deficient mice are less prone to NAFLD[9]. Yet, deficiency of TNF receptors does not prevent elevation of serum ALT in ob/ob mice[10] or after intragastric overfeeding of a high-fat diet[11]. TNF-α can induce insulin resistance. This implies that TNF-α and other pro-inflammatory cytokines may have a role in NAFLD but are not the sole cause of the same.

Hepatic fat deposition with hepatocellular damage, a feature of NAFLD, may also be mediated by pro-inflammatory prostaglandins (PGs). Among the more than twenty isozymes of mammalian PLA_2, group IVA PLA_2 (IVAPLA2) is a key enzyme responsible for the release of arachidonic acid (AA), a precursor of PGs. IVA-PLA_2-knockout mice fed normal chow diets showed a decrease in hepatic triacylglycerol content and the size of epididymal adipocytes was smaller with a lower serum level of pro-inflammatory prostaglandin E_2 (PGE_2) compared with wild-type mice, suggesting that the circulating level of PGE_2 is related to the levels of intracellular triglyceride (TG) in the liver and adipose tissues[12]. Stimulation of rat hepatocytes with PGE_2 and the administration of PGE_2 to rats induced increases in TG level in the cells and the liver, respectively[13,14], suggesting that IVA-PLA_2 mediates fat deposition in the liver and adipose tissues through the generation of PGs that are pro-inflammatory in nature and thus, could predispose to the development of NAFLD. A deficiency of IVA-PLA_2 alleviated fatty liver damage caused by high-fat diets[15] as a result of the lower generation of IVA-PLA_2 metabolites, such as PGE_2 that has pro-inflammatory action. Thus, NAFLD is a low-grade systemic inflammatory condition.

COULD NAFLD BE A RESULT OF DEFICIENCY OF ANTI-INFLAMMATORY CYTOKINES AND BIOACTIVE LIPIDS?

Fatty livers of obese *fa/fa* rats are vulnerable to injury when challenged by insults such as endotoxin, ischemia-reperfusion or acute ethanol treatment that could lead to NAFLD. When obese *fa/fa* rats and their lean littermates were fed a diet low in fat (12% of total calories) or a diet with 60% calories as lard for 8 wk, hyperglycemia and steatohepatitis occurred in the *fa/fa* rats fed the high-fat diet. This was accompanied by liver injury as evidence by enhanced levels of hepatic enzymes (such as alanine aminotransferase) that was found to be associated with increased TNF-α and TGF-β, collagen deposition, up-regulation of α-smooth muscle actin, increased TIMP1 (a component of family of tissue inhibitors of metalloproteinases), and elevated oxidative stress, lipid peroxides, protein carbonyls and reduced glutathione and antioxidant enzymes in the *fa/fa* rats fed with the high-fat diet[16]. Despite the fact that inflammatory events play a significant role in NAFLD, relatively little attention has been paid to anti-inflammatory events.

It is possible that enhanced IL-6, TNF-α, PGE_2 levels and insulin resistance seen in NAFLD could be due to a deficiency of anti-inflammatory molecules. For instance, AA released by IVA-PLA_2 can form a precursor to anti-inflammatory bioactive lipids such as lipoxins (LXs), resolvins and protectins that suppress IL-6, TNF-α and PGE_2 production and ameliorate insulin resistance[17-19]. This is supported by the observation that enteral and intravenous supplementation of omega-3 fatty acids can ameliorate hepatic steatosis in a murine model of NAFLD[20]. In addition, a relative depletion in polyunsaturated fatty acids (PUFAs), particularly of the *n*-6 and *n*-3 series in hepatic triacylglycerols and of the *n*-3 series in liver phospholipids, with decreased 20:4, *n*-6/18:2, *n*-6 and (20:5, *n*-3 + 22:6, *n*-3)/18:3, *n*-3 ratios with simultaneously higher *n*-6/*n*-3 ratios in liver and adipose tissue, 18:1, *n*-9 *trans* contents in adipose tissue, and hepatic lipid peroxidation and protein oxidation indexes was reported in NAFLD patients[21]. These results suggest that an alteration in the metabolism of both *n*-6 and *n*-3 fatty acids occur in NAFLD.

Thus, it is likely that an imbalance between the pro- and anti-inflammatory molecules that is tilted more in favor of the former could trigger the development of NAFLD. Hence, I propose that failure to produce adequate amounts of anti-inflammatory molecules such as LXs, resolvins and protectins and cytokines IL-4 and IL-10 play a role in the pathobiology of NAFLD.

ESSENTIAL FATTY ACID METABOLISM

In view of the proposal that altered metabolism of EFAs in the form of enhanced formation of pro-inflammatory eicosanoids and decreased formation of anti-inflammatory bioactive lipids, especially those from ω-3 fatty acids, play a significant role in NAFLD, a close look at the metabolism of EFAs is necessary.

Cis-linoleic acid (LA, 18:2 ω-6) and α-linolenic acid (ALA, 18:3 ω-3) are EFAs. LA is converted to γ-linolenic acid (GLA, 18:3, ω-6) by the action of the enzyme Δ^6 desaturase and GLA is elongated to form di-homo-GLA (DGLA, 20:3, ω-6), the precursor of the 1 series of prostaglandins. DGLA can be converted to AA (20:4, ω-6)

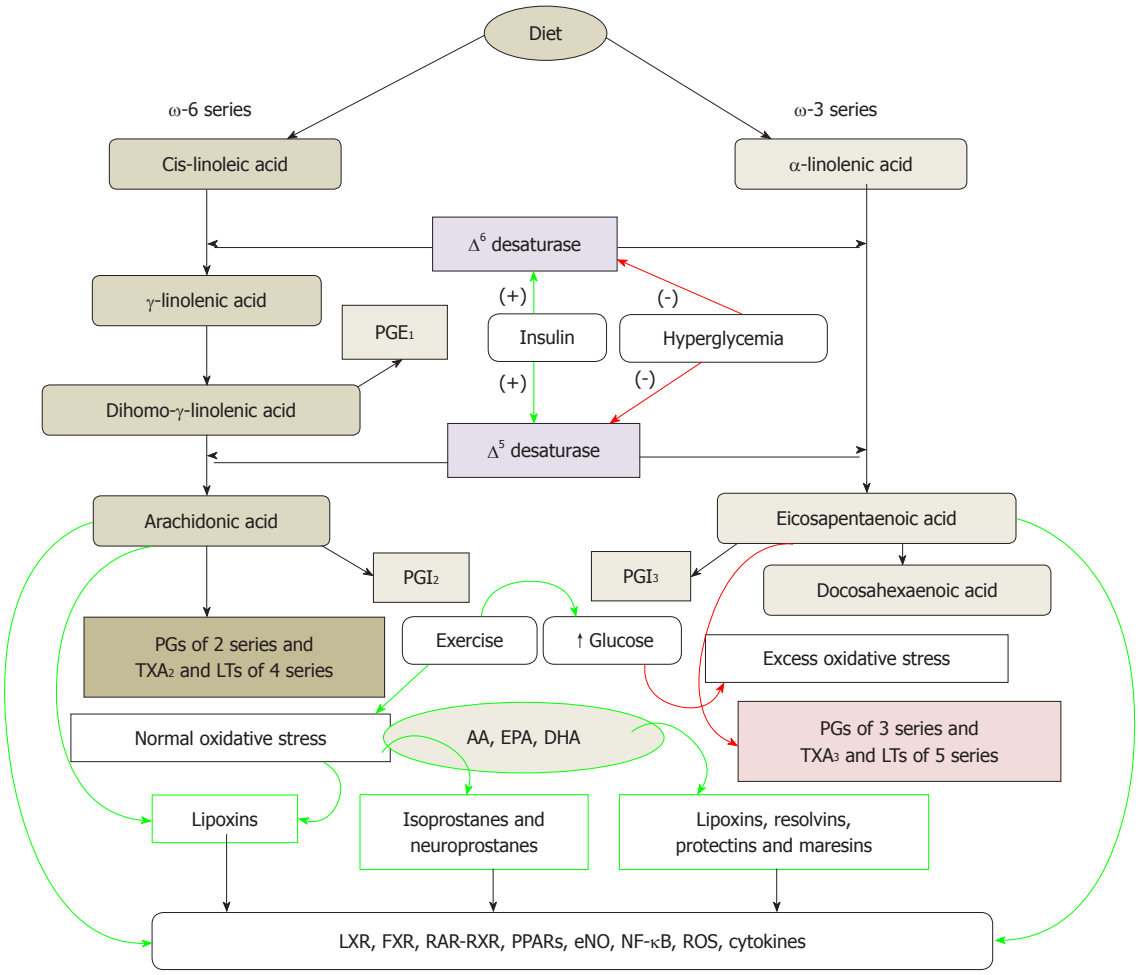


Figure 1 Metabolism of essential fatty acids and their modulation by insulin, glucose, exercise and oxidative stress. Calorie restriction increases the activity of Δ^6 and Δ^5 desaturases. High-fat diet, *trans*-fats and cholesterol block the activities of Δ^6 and Δ^5 desaturases. (-): Inhibition of synthesis or action; (+): Enhancement of synthesis or action. Green arrows indicate beneficial action and/or anti-inflammation whereas red arrows indicate harmful action and/or pro-inflammation. PG: Pro-inflammatory prostaglandin; AA: Arachidonic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; LTs: Leukotrienes.

by the action of the enzyme Δ^5 desaturase. AA forms the precursor of 2 series of prostaglandins, thromboxanes and the 4 series LTs. ALA is converted to eicosapentaenoic acid (EPA, 20:5, ω-3) by Δ^6 and Δ^5 desaturases. EPA forms the precursor of the 3 series of prostaglandins and the 5 series of Leukotrienes (LTs). EPA can be elongated to form docosahexaenoic acid (DHA, 22:6, ω-3). AA, EPA and DHA also form precursors to a group of novel compounds: LXs, resolvins, protectins and maresins[22-32] that have anti-inflammatory action (Figure 1). Eicosanoids bind to G protein-coupled receptors on many cell types and mediate virtually every step of inflammation, are found in inflammatory exudates and their synthesis is increased at sites of inflammation. Non-steroidal anti-inflammatory drugs such as aspirin inhibit cyclooxygenase (COX) activity and thus, are believed to bring about their anti-inflammatory action.

LXS, RESOLVINS, PROTECTINS AND MARESINS

There are two COX enzymes, the constitutively expressed COX-1 and the inducible enzyme COX-2. Different types of PGs are formed by the action of COX enzymes depending on the substrate fatty acid from which they are derived.

There are 3 types of lipoxygenases and are present in only a few types of cells. 5-lipoxygenase (5-LO), present in neutrophils, produces 5-hydroxyeicosatetraenoic acid (5-HETE), which is chemotactic for neutrophils and is converted into LTs. LTB_4, a potent chemotactic and activator of neutrophils, induces aggregation and adhesion of leukocytes to vascular endothelium, generation of reactive oxygen species and release of lysosomal enzymes. The cysteinyl-containing leukotrienes C_4, D_4, and E_4 (LTC_4, LTD_4 and LTE_4) induce vasoconstriction, bronchospasm and vascular permeability in venules. LTs are more potent than histamine in increasing vascular permeability and causing bronchospasm. LTs mediate their actions by binding to cysteiny leukotreine 1 (CysLT1) and CysLT2 receptors. In general, PGs, LTs and thromboxanes (TXs) formed from DGLA and AA are pro-inflammatory in nature. PGs, TXs and LTs formed from EPA also have pro-inflammatory action but are generally

less pro-inflammatory compared to those formed from AA.

AA, EPA and DHA also form precursors to potent anti-inflammatory compounds: LXs, resolvins, protectins and maresins. LXs are generated from AA, EPA and DHA (LXA_4 is formed from AA; LXA_5 is formed from EPA; resolvins are formed from EPA and DHA and protectins from DHA; and all these products have potent anti-inflammatory actions) by transcellular biosynthetic mechanisms involving two cell populations. Neutrophils produce intermediates in LX synthesis and these are converted to LXs by platelets interacting with leukocytes. LXA_4 and LXB_4 are generated by the action of platelet 12-LO on neutrophil-derived LTA_4. LXs inhibit leukocyte recruitment, neutrophil chemotaxis and adhesion to endothelium[28]. LXs have a negative regulation on LT synthesis and action and help in the resolution of inflammation. An inverse relationship generally exists between LXs and LTs and the balance between these two molecules appears to be crucial in the determination of degree of inflammation and its final resolution[22,25,27,30-32].

ASPIRIN-TRIGGERED 15 EPIMER LXS, RESOLVINS AND PROTECTINS

The formation of aspirin-triggered 15 epimer LXs are potent counter regulators of polymorphonuclear neutrophils (PMNs)-mediated injury and acute inflammation. Acetylated COX-2 enzyme of endothelial cells generates 15R-HETE from AA that is converted by activated PMNs to the 15-epimeric LXs that have potent anti-inflammatory properties[23-32]. This cross-talk between endothelial cells and PMNs leading to the formation of 15R-HETE and its subsequent conversion to 15-epimeric LXs by aspirin-acetylated COX-2 is a protective mechanism to prevent local inflammation on the vessel wall by regulating the motility of PMNs, eosinophils and monocytes[30-32]. Endothelial cells also oxidize AA, EPA and DHA *via* P450 enzyme system to form various hydroxyeicosatetraenoic acids and epoxyeicosatrienoic acids such as 11,12-epoxy-eicosatetraenoic acid(s) that have many biological actions that include blocking endothelial cell activation, while non-enzymatic oxidation products of EPA inhibit phagocyte-endothelium interaction and suppress the expression of adhesion molecules[33-38].

Akin to the formation of 15R-HETE and 15-epimeric LXs from AA, similar compounds are also formed from EPA and DHA. In the presence of aspirin, activated COX-2 of human endothelial cells converts EPA to 18R-HEPE, 18-HEPE and 15R-HEPE. Activated human PMNs, in turn, converts 18R-HEPE to 5,12,18R-triHEPE and 15R-HEPE to 15-epi-LXA_5 by their 5-LO. Both 18R-HEPE and 5,12,18R-triHEPE inhibited LTB_4-stimulated PMN transendothelial migration. 5,12,18R-triHEPE effectively competed with LTB_4 for its receptors and inhibited PMN infiltration suggesting that it suppresses LT-mediated responses at the sites of inflammation[22,25,27,31,32,39,40].

The conversion of EPA by human endothelial cells with upregulated COX-2 treated with ASA of EPA to 15-epi-LX, also termed aspirin-triggered LX (ATL), and to 18R-HEPE and 15R-HEPE is interesting. These compounds in turn, are used by polymorphonuclear leukocytes to generate separate classes of novel trihydroxy-containing mediators, including 5-series 15R-LX(5) and 5,12,18R-triHEPE, which are potent inhibitors of human polymorphonuclear leukocyte transendothelial migration and infiltration *in vivo* (ATL analogue > 5,12,18R-triHEPE > 18R-HEPE). Acetaminophen and indomethacin also permitted 18R-HEPE and 15R-HEPE generation with recombinant COX-2. The formation of these bioactive lipid mediators *via* COX-2-nonsteroidal anti inflammatory drug-dependent oxygenations and cell-cell interactions may have significant therapeutic benefits in inflammation[17,27,31,32,39,40].

Leukocytes, brain and glial cells transform enzymatically DHA to 17R series of hydroxy DHAs that, in turn, is converted enzymatically to di- and tri-hydroxy containing docosanoids[31,32,40-42]. The conversion of DHA to 17S-hydroxy-containing docosanoids denoted as docosatrienes (the main bioactive member of the series was 10,17S-docosatriene) and 17S series resolvins serve as regulators of both leukocytes reducing infiltration *in vivo* and glial cells blocking their cytokine production. Thus, DHA is the precursor to novel docosatrienes and 17S series resolvins that have anti-inflammatory action and resolve inflammation.

Similar small molecular weight compounds are also generated from AA, EPA and DHA: 15R-hydroxy containing compounds from AA, 18R series from EPA and 17R-hydroxy series from DHA. All these compounds have potent anti-inflammatory actions, resolve inflammation and hence are called "resolvins". Resolvins inhibit cytokine generation, leukocyte recruitment, leukocyte diapedesis and exudate formation. The formation of resolvins from AA, EPA and DHA from acetylated COX-2 are generated *via* transcellular biosynthesis (e.g. due to cell-cell communication between endothelial cells and PMNs) and their main purpose appears to be to suppress inflammation. Resolvins inhibit brain ischemia-reperfusion injury[31,32,40-42]. It is likely that LXs, resolvins and protectins (docosanoids are also called as protectins since they have neuroprotective actions) serve as endogenous anti-inflammatory and cytoprotective molecules[17,18,28]. The general cytoprotective properties that have been attributed to AA, EPA and DHA can be related to their conversion to LXs, resolvins and docosanoids (protectins) (Figures 2-5). Hence, defects in the formation and action of LXs, resolvins and protectins could lead to perpetuation of inflammation[31,32].

ANTI-INFLAMMATORY MOLECULE LXA_4 IS DETECTABLE IN URINE

LXA_4, generated by LO transformation of AA possess potent anti-inflammatory activity *in vivo* and tempo-

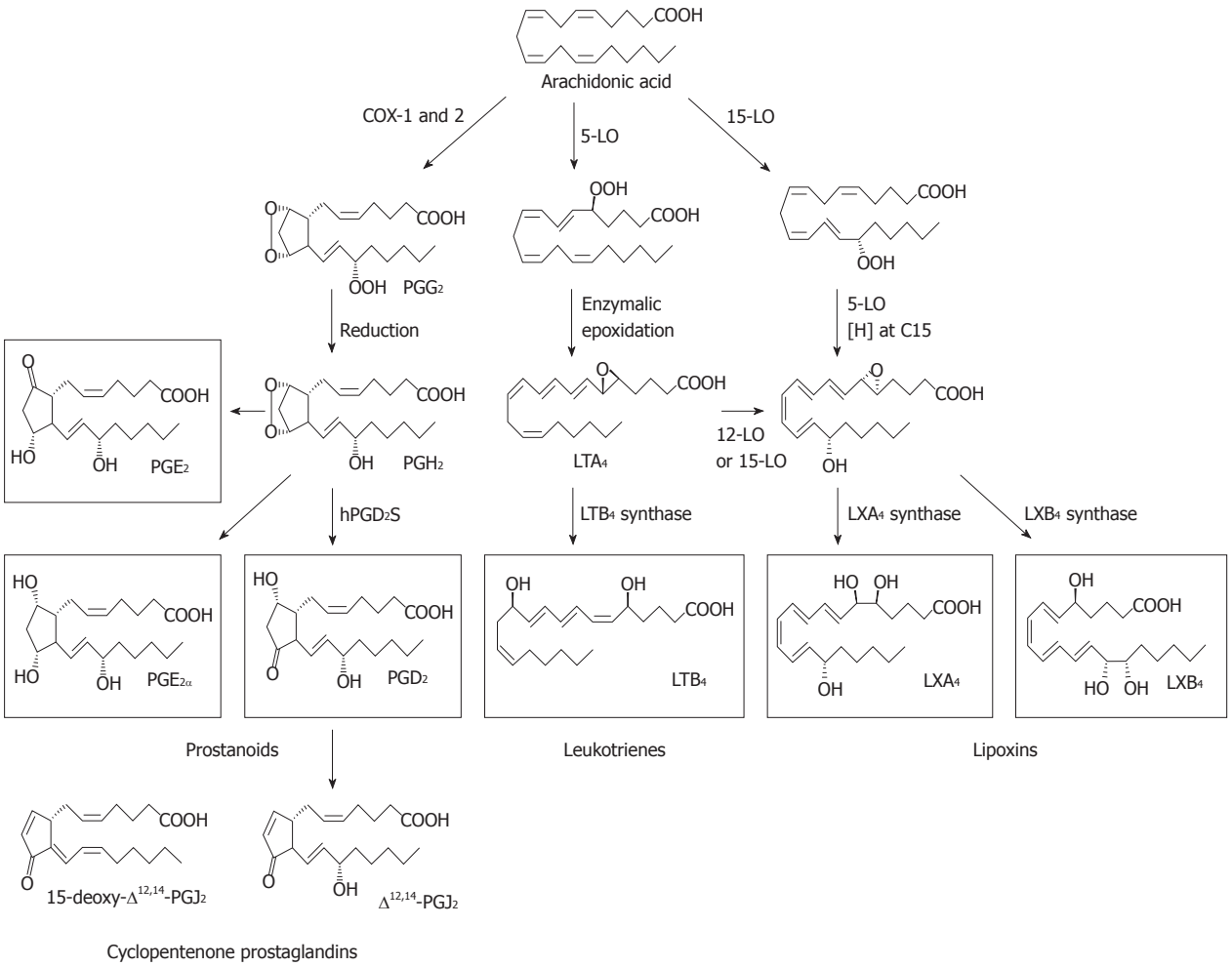


Figure 2 Metabolism of arachidonic acid showing different metabolites formed from it. LO: Lipoxygenase; LXs: Lipoxins; LTs: Leukotrienes; PG: Pro-inflammatory prostaglandin.

ral biosynthesis of LXs, concurrent with spontaneous resolution, has been observed during exudate formation[29-31,42-44]. LXs, resolvins, protectins and maresins are detectable in the plasma[17,31,32]. Recently, it was reported that urine from healthy subjects contains LXA_4[45] and strenuous exercise significantly increased its urinary excretion in healthy volunteers[46], suggesting that alterations in the urinary excretion of LXA_4 can used as a reflection of changes in its (LXA_4) formation to monitor changes in the inflammatory events/diseases. It is possible that other anti-inflammatory bioactive lipids such as resolvins, protectins and maresins may also be detectable in the urine. Since urinary levels of LXA_4 was decreased (similar decrease may occur of other bioactive lipids such as resolvins, protectins and maresins) while that of cysteinyl leukotrienes (cysLTs) increased in volunteers aged from 26 to over 100 years, leading to a profound unbalance of the LXA(4)/cysLTs ratio, that may serve as an index of the endogenous anti-inflammatory potential[47]. Hence, measurement of urinary and plasma levels of LXs, resolvins, protectins, maresins and leukotrienes could be used to monitor the inflammatory process that occurs in various diseases including NAFLD.

ALTERED EFA METABOLISM IN NAFLD IN THE FORM OF A DECREASE IN ANTI-INFLAMMATORY AND AN INCREASE IN PRO-INFLAMMATORY BIOACTIVE LIPIDS

NAFLD consists of a variety of pathological states ranging from the simple buildup of fat in the liver (hepatic steatosis) to nonalcoholic steatohepatitis, cirrhosis and ultimately liver failure[48-51]. Current statistics suggest that NAFLD is a major cause of liver-related morbidity and mortality and is believed to account for approximately 80% of individuals with elevated serum liver enzymes[52] and further to that, up to 30% of the Western population may have NAFLD[53]. NAFLD is associated with metabolic disorders such as obesity[54] and diabetes[55], as well as with prolonged chemotherapy[56] and total parenteral nutrition[57-59]. In view of such wide spread incidence and prevalence of NAFLD, it is important to understand its etiopathogenesis to develop suitable remedial measures.

Intravenous administration of fish oil that is rich in ω-3 fatty acids EPA and DHA reduced parenteral nutrition-induced cholestasis in newborn piglets[60] and

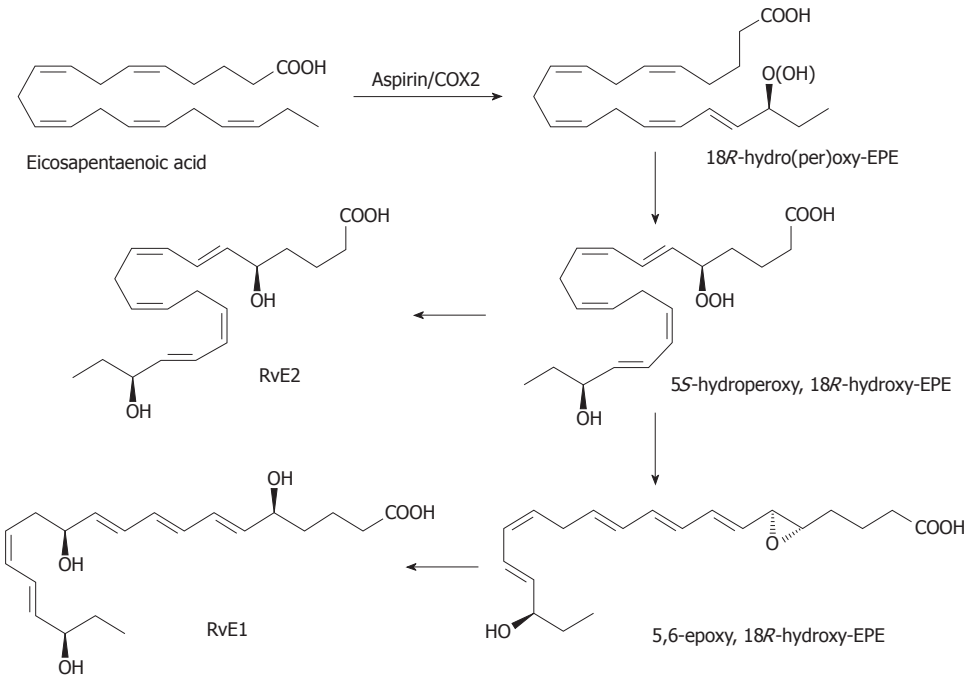


Figure 3 Scheme showing the formation of resolvin E derived from eicosapentaenoic acid. In the endothelial cells, the **cyclo-oxygenase (COX)-2 enzyme that** has been acetylated introduces an 18*R* hydroperoxy-group into the eicosapentaenoic acid molecule (c.f. the role of aspirin in the biosynthesis of the epi-lipoxins). This is reduced to the corresponding hydroxy compound before a 5*S*-hydroperoxy group is introduced into the molecule by the action of 5-lipoxygenase as in the biosynthesis of leukotrienes. A further reduction step produces 15S,18R-dihydroxy-EPE or resolvin E2. Alternatively, the 5*S*-hydrpperoxy, 18*R*-hydroxy-EPE intermediates is converted to a 5,6-epoxy fatty acids in polymorphonuclear leukocytes I humans and eventually to 5*S*,12*R*,18*R*-trihydroxy-6*Z*,8*E*,10*E*,14*Z*,16*E*-eiocsapentaenoic acid or resolvin E1 by process similar to the formation of leukotrienes in leukocytes.

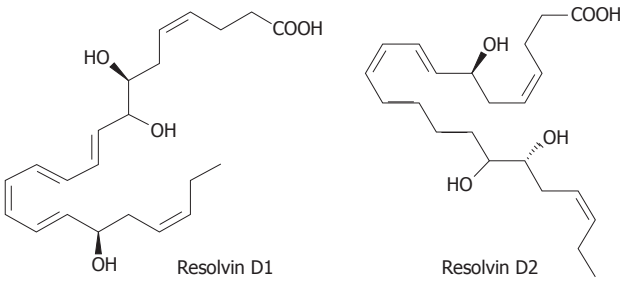


Figure 4 Structures of Resolvin D1 and D2. DHA is converted to 17R-resolvins by a similar aspirin-triggered mechanism similar to the scheme shown in Figure 2. In the absence of aspirin, COX-2 of endothelial cells converts DHA to 13S-hydroxy-DHA. In the presence of aspirin, the initial product is 17R-hydroxy-DHA, which is converted to 7S-hydroperoxy, 17R-hydroxy-DHA by the action of a lipoxygenase, and thence via an epoxy intermediate to epimeric resolvins D1 and D2. An alternative lipoxygenase-generated intermediate, 4S-hydroperoxy, 17R-hydroxy-DHA, is transformed *via* an epoxide to epimeric resolvins D3 and D4. 17S Resolvins of the D series are produced in cells in the absence of aspirin by a reaction catalyzed in the first step by a lipoxygenase. **COX: Cyclo-**oxygenase; **DHA: Docosahexaenoic acid.**

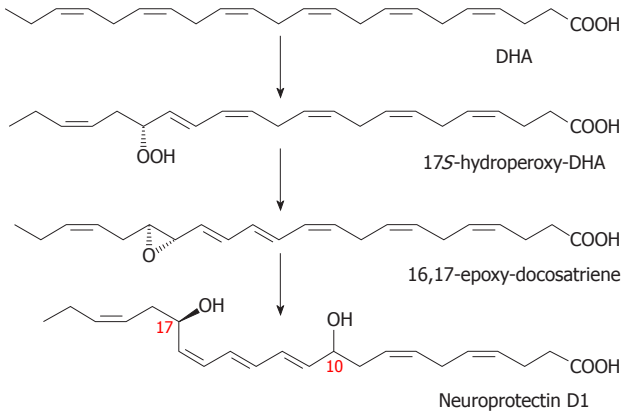


Figure 5 Scheme showing the synthesis of neuroprotectin D1. Resolvins are generated in brain tissue in response to aspirin treatment and in addition, docosatrienes termed neuroprotectins are also produced. The lipoxygenase product 17*S*-hydroperoxy-DHA is converted first to a 16(17)-epoxide and then to the 10, 17-dihydroxy docosatriene denoted as 10, 17 *S*-DT or NPD1. As with the leukotrienes, there are three double bonds in conjugation, hence the term "triene", although there are six double bonds in total. Figures 2-5 **are** from[27,31,32,64,119]. **DHA: Docosahexaenoic acid.**

rats[61,62] and dietary ω-3 and ω-6 PUFAs have the ability to regulate hepatic lipogenesis by reducing sterol regulatory element-binding protein-1 in the liver[63,64]. In a clinical study, wherein analysis of liver and abdominal adipose tissue fatty acids was carried out in normal controls and those with NAFLD, it was noted that NAFLD patients had a depletion in PUFAs of the ω-6 and ω-3 series in liver triacylglycerols, with decreased AA/LA and EPA + DHA/ALA ratios, whereas liver phospholipids contained higher ω-6 and lower ω-3 PUFAs[21]. These findings were accompanied by an enhancement of (1) ω-6/ω-3 ratio in liver and adipose tissue; (2) **18:1,** ω-9 *trans* levels in adipose tissue; **and (3) hepatic lipid peroxidation and protein** oxidation indexes. These results suggest that a marked enhancement in PUFA ω-6/ω-3 ratio occurs in the liver of NAFLD patients. Based on these results, it was suggested that depletion of hepatic PUFA content may re-

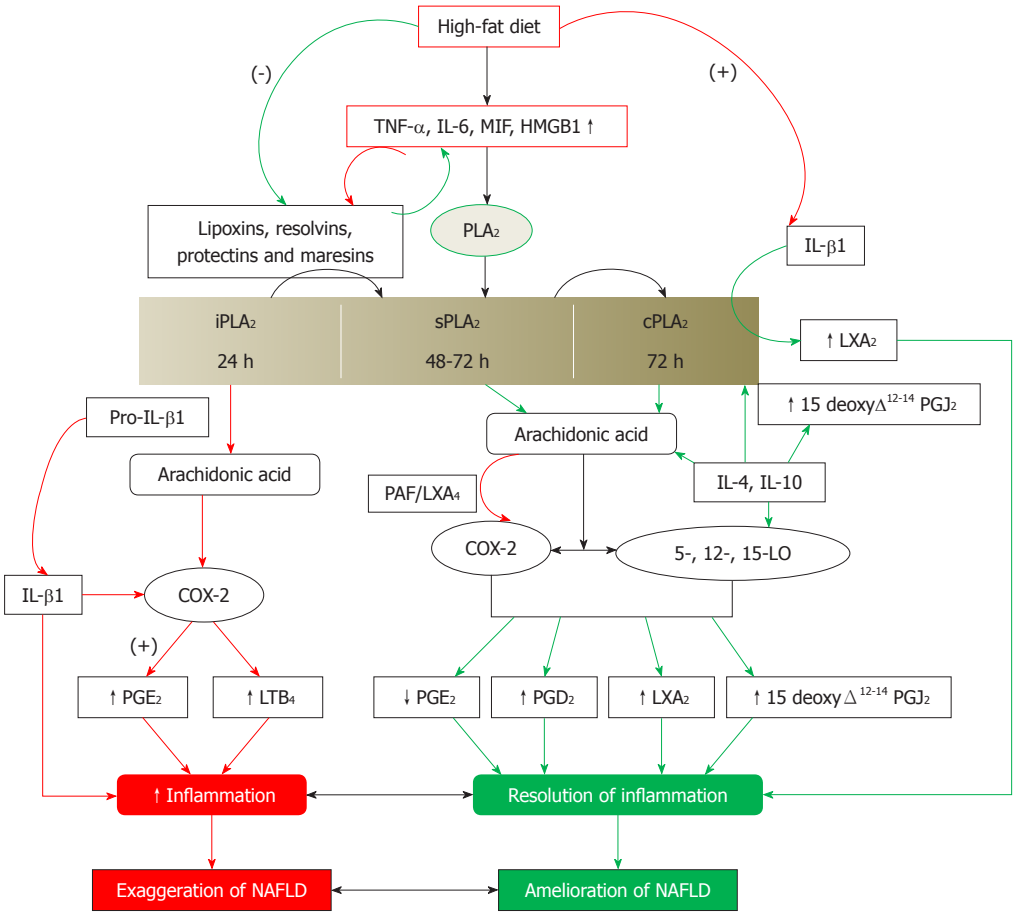


Figure 6 Scheme showing the role of eicosanoids, lipoxins, resolvins, protectins and maresins in resolution of non-alcoholic fatty liver disease. Green arrows indicate events that will lead to resolution of non-alcoholic fatty liver disease (NAFLD). Red arrows indicate events that will lead to initiation and/or progression of NAFLD. (-): Inhibition or suppression of action; (+): Activation or enhancement of action; TNF-α: Tumor necrosis factor-α; LXs: Lipoxins; COX: Cyclo-oxygenase; LO: Lipoxygenase; PG: Pro-inflammatory prostaglandin. For further details see[82-90,119].

sult from both defective desaturation of EFAs (both LA and ALA) that could be due to both inadequate intake of EFAs (both LA and ALA) and higher intake of the 18:1, ω-9 *trans* isomer leading to desaturase inhibition and, possibly, from an increased peroxidation of PUFAs due to oxidative stress[21].

Prolonged use of total parenteral nutrition can lead to nonalcoholic fatty liver disease, ranging from hepatic steatosis to cirrhosis and liver failure. Mice that receive fat-free, high-carbohydrate diet develop severe liver damage as determined by histology and magnetic resonance spectroscopy as well as elevation of serum liver function tests. In such a murine model of NAFLD in which all animals develop steatosis and liver enzyme disturbances, intravenous administration of ω-3 fatty acid emulsion attenuated NAFLD and prevented hepatic pathology and normalized liver function tests[20], suggesting that ω-3 fatty acids protect liver against injury including NAFLD. In addition, NAFLD is associated with low levels of adiponectin and relatively high levels of TNF-α[65]. This lends support to the proposal that NAFLD could be an inflammatory condition and methods designed to suppress inflammation could be of significant benefit. In addition, adiponectin antagonizes both the production and activity of TNF-α, whereas TNF-α inhibits adiponectin. Adiponectin acts directly on hepatocytes to inhibit fatty acid synthesis and uptake while stimulating fatty acid oxidation. Thus, it is likely that a combination of low adiponectin and high TNF-α levels in the context of increased hepatic exposure to free fatty acids results in hepatic steatosis, severe hepatic insulin resistance and ultimately NAFLD[65].

It is interesting to note that PUFAs, especially ω-3 EPA and DHA and ω-6 AA, DGLA and GLA and their products such as PGE_1, LXs, resolvins, protectins and maresins suppress the production of IL-6, TNF-α and MIF that are pro-inflammatory cytokines, free radical generation and lipid peroxidation process[22-32,39-44,64,66-73]. Furthermore, insulin resistance itself may perpetuate NAFLD since insulin has anti-inflammatory actions[74-78], whereas exercise is beneficial since it (exercise) is anti-inflammatory in nature[79,80]. In the initial stages of exercise, there will be an increase in the production of IL-6 that triggers elevation in the production of endogenous anti-oxidants such as superoxide dismutase[81] and LXA_4. In addition, exercise enhances the production of LXA_4 that may explain its anti-inflammatory action[81]. Thus, there is a close relationship that exists between high fat diet, EFA/PUFA metabolism, pro-inflammatory cytokines, insulin resistance, insulin and exercise (Figure 6).

NAFLD is a low-grade systemic inflammatory condition. Increased formation of pro-inflammatory cytokines and eicosanoids and/or reduced formation of anti-inflammatory cytokines and inflammation resolving bioactive lipids may participate in the pathobiology of NAFLD. Thus, release and timely formation of anti-inflammatory bioactive lipids is necessary to prevent NAFLD and/or resolution of inflammation seen in NAFLD. The release and formation of anti-inflammatory bioactive lipids depends on the activity of phospholipase A_2. This scheme is applicable to both acute and chronic inflammation. NAFLD that starts may initially have an acute inflammatory component and will become chronic due to the changes in the activities of various sub-classes of phospholipase A_2 and continued exposure to pro-inflammatory stimuli such as high-fat diet.

There are three classes of phospholipases that control the release of AA and other PUFAs: calcium-independent PLA_2 ($iPLA_2$), secretory PLA_2 ($sPLA_2$) and cytosolic PLA_2 ($cPLA_2$)[82]. Each class of PLA_2 is further divided into isoenzymes for which there are 10 for mammalian $sPLA_2$, at least 3 for $cPLA_2$ and 2 for $iPLA_2$. During the early phase of inflammation, COX-2 derived PGs and TXs and lipoxygenase-derived LTs initiate exudate formation and inflammatory cell influx[83]. TNF-α causes an immediate influx of neutrophils concomitant with PGE_2 and LTB_4 production, whereas during the phase of resolution of inflammation, an increase in LXA_4 (LX A_4), PGD_2 and its product 15deoxy$\Delta^{12\text{-}14}PGJ_2$ formation occurs that induces resolution of inflammation with a simultaneous decrease in PGE_2 synthesis that stops neutrophil influx and enhances phagocytosis of debris[84,85]. Thus, there appears to be two waves of release of AA and other PUFAs: one at the onset of inflammation that causes the synthesis and release of PGE_2 and a second at resolution for the synthesis of anti-inflammatory PGD_2, 15deoxy$\Delta^{12\text{-}14}PGJ_2$, and LXs that are necessary for the suppression of inflammation. Thus, COX-2 enzyme has both harmful and useful actions by virtue of its ability to give rise to pro-inflammatory and anti-inflammatory PGs and LXs.

Increased type VI $iPLA_2$ protein expression was found to be the principal isoform expressed from the onset of inflammation up to 24 h, whereas type Ⅱa and V $sPLA_2$ was expressed from the beginning of 48 h till 72 h while type Ⅳ $cPLA_2$ was not detectable during the early phase of acute inflammation but increased progressively during resolution, peaking at 72 h. This increase in type Ⅳ $cPLA_2$ was mirrored by a parallel increase in *COX-2* expression[86]. The increase in $cPLA_2$ and COX-2 occurred in parallel, suggesting a close enzymatic coupling between these two. Thus, there is a clear-cut role for different types of PLA_2 in distinct and different phases of inflammation. Selective inhibition of $cPLA_2$ resulted in the reduction of pro-inflammatory molecules PGE_2, LTB_4, IL-1β and platelet-activating factor (PAF). Furthermore, inhibition of types Ⅱa and V $sPLA_2$ not only decreased PAF and LXA_4 (LX A_4) but also resulted in a reduction in $cPLA_2$ and COX-2 activities. These results suggest that $sPLA_2$-derived PAF and LXA_4 induce COX-2 and type Ⅳ $cPLA_2$. IL-1β induced $cPLA_2$ expression. This suggests that one of the functions of IL-1 is not only to induce inflammation but also to induce $cPLA_2$ expression to initiate resolution of inflammation[87,88].

Synthetic glucocorticoid dexamethasone inhibited both $cPLA_2$ and $sPLA_2$ expression, whereas type Ⅳ $iPLA_2$ expression is refractory to its suppressive actions[89-91]. Activated $iPLA_2$ contributes to the conversion of inactive proIL-1β to active IL-1β, which in turn induces $cPLA_2$ expression that is necessary for resolution of inflammation.

LXs, especially LXA_4 inhibit TNF-α-induced production of ILs, promote TNF-α mRNA decay, TNF-α secretion and leukocyte trafficking and thus attenuated inflammation. Based on these evidences, in NAFLD there could occur increased formation of PGE_2 and other pro-inflammatory eicosanoids and decreased production of PGD_2, 15 deoxy$\Delta^{12\text{-}14}$ PGJ_2, LXs, resolvins, protectins and maresins that have anti-inflammatory action. Defective function of $sPLA_2$ and $cPLA_2$, as a result of which decreased release of AA, EPA and DHA could occur that, in turn, leads to reduced formation of pro-resolving and anti-inflammatory PGD_2, 15 deoxy$\Delta^{12\text{-}14}$ PGJ_2, LXs, resolvins, protectins and maresins leads to the initiation and perpetuation of NAFLD.

It is noteworthy that high-fat diet, *trans*-fats and cholesterol interfere with EFA metabolism by blocking the actions of Δ^6 and Δ^5 desaturases and thus, decrease the levels of GLA, DGLA, AA, EPA and DHA[25,27,32,64]. As a result, the formation of anti-inflammatory bioactive lipids, PGE_1, 15 deoxy$\Delta^{12\text{-}14}$ PGJ_2, LXs, resolvins, protectins and maresins, will also be decreased due to substrate deficiency that leads to initiation and perpetuation of the inflammatory process[31,32,64]. On the other hand, insulin and diet restriction enhance the action of Δ^6 and Δ^5 desaturases[25,27,92], augment tissue levels of GLA, DGLA, AA, EPA and DHA that leads to increased formation of PGE_1, 15 deoxy$\Delta^{12\text{-}14}$ PGJ_2, LXs, resolvins, protectins and maresins that could ameliorate inflammation and NAFLD.

DEFECT IN THE ACTIVITY OF Δ^6 AND Δ^5 DESATURASES AND ANTI-INFLAMMATORY BIOACTIVE LIPIDS IN NAFLD

Based on the preceding discussion, it is proposed that patients with NAFLD have low plasma and hepatic levels of AA, EPA and DHA due to decreased Δ^6 and Δ^5 desaturases, reduced formation of PGD_2, 15 deoxy$\Delta^{12\text{-}14}$ PGJ_2, LXs, resolvins, protectins and maresins as a result of substrate deficiency, and a defect in the production of adequate amounts of anti-inflammatory cytokines IL-4, IL-10 and significantly higher levels of pro-inflammatory molecules PGE_2, TXs, LTs and cytokines IL-6, and TNF-α compared to normal healthy controls. It is also

possible that there may be a direct correlation between the concentrations of pro-inflammatory and anti-inflammatory molecules and the degree of NAFLD.

In a study, patients with dyslipidemia (Fredrickson type Ⅱb), who had asymptomatic persistent transaminasemia lasting 24 wk and evidence of hepatic fat infiltration on ultrasonography and liver biopsy, were studied with regard to the efficacy and safety of ω-3 fatty acids (rich in EPA and DHA), atorvastatin, an HMG-CoA reductase inhibitor, and orlistat, which inhibits both gastric and pancreatic lipases in the enteric lumen, and it was found that all three drugs were effective[93]. It may be mentioned here that both ω-3 fatty acids EPA and DHA and HMG-CoA reductase inhibitors are known to have anti-inflammatory actions (reviewed in 25, 27, 32) that may explain their beneficial action in NAFLD. The role of PUFAs in NAFLD is further supported by the observation that reductions in AA in free fatty acids, triacylglycerol and phosphatidylcholine and a decrease in EPA and DHA in diacylglycerol fractions in the liver biopsy specimens occurred[94]. These results reiterate the proposal that deficiency of AA, EPA and DHA occurs even in the hepatic tissue in NAFLD.

Further support to the role of PUFAs and their pro- and anti-inflammatory products and pro- and anti-inflammatory cytokines in the pathobiology of NAFLD is derived from studies performed using mice in which 12/15-LO gene (Alox 15) has been disrupted. 12/15-LO is a member of the lipoxygenase family that converts AA into lipid mediators such as 12-HETE and 15-HETE[95]. 12/15-LO products have pro-inflammatory actions and activate nuclear factor κB and c-Jun amino-terminal kinase and stimulate the expression of proinflammatory cytokines[96,97]. It is known that 12/15-LO plays an important role in the metabolic syndrome, which is a low-grade systemic inflammatory condition[98-104]. Disruption of **gene encoding for 12/15-LO (Alox15) in mice** delayed the onset of atherosclerosis[105,106], were resistant to the development of streptozotocin-induced and autoimmune diabetes[107,108] and protected from high-fat diet-induced obesity and metabolic consequences, including adipose tissue inflammation and insulin resistance[109,110]. Conversely, transgenic mice overexpressing 12/15-LO in cardiomyocytes displayed exacerbated cardiac inflammation and fibrosis and more advanced heart failure[111].

In the *ob/ob* mice, an obesity model of insulin resistance and fatty liver disease, supplementation of EPA and DHA improved insulin-sensitivity in adipose tissue and liver, upregulated hepatic PPAR-γ, glucose transport (GLUT-2/GLUT-4) and insulin receptor signaling (*IRS-1/IRS-2*) genes, increased adiponectin levels and induced AMPK phosphorylation, a fuel-sensing enzyme and a gatekeeper of the energy balance. Hepatic steatosis was alleviated by ω-3-PUFAs in this animal model as a result of increased formation of resolvin E1 and protectin D1. Both resolvin E1 and protectin D1 mimicked the insulin-sensitizing and antisteatotic effects of ω-3-PUFAs and induced adiponectin expression[112]. These results lend direct support to the proposal that ω-3 PUFAs and their anti-inflammatory products LXs and resolvins prevent obesity-induced insulin resistance and NAFLD. Furthermore, obese subjects who are more prone to develop NAFLD are known to have decreased hepatic Δ^6 and Δ^5 desaturase activity[113] that may, in turn, lead to decrease in the hepatocyte content of AA, EPA and DHA that form precursors to anti-inflammatory LXs, resolvins and protectins. Park *et al*[114] showed that long-term use of ezetimibe (for 24 mo), a lipid-lowering drug, significantly improved metabolic parameters including visceral fat area, fasting insulin, homeostasis model assessment of insulin resistance, triglycerides, total cholesterol, low-density lipoprotein cholesterol, oxidative-LDL, the net electronegative charge modified-LDL, profiles of lipoprotein particle size and fatty acids component, estimated desaturase activity and lowered serum alanine aminotransferase and high-sensitivity C-reactive protein levels in patients with NAFLD. It is noteworthy that these patients also showed an increase Δ^5 desaturase activity indicating that plasma and hepatic levels of AA, EPA and DHA, the precursors of LXs, resolvins and protectins, have increased. On the other hand, Fujita *et al*[115] showed that antiplatelet drugs, aspirin, ticlopidine and cilostazol, significantly attenuated liver steatosis, inflammation and fibrosis in the Fisher 344 male rats that were given a choline-deficient, l-amino acid-defined (CDAA) diet with high-fat high-calorie diet that induced NAFLD. It may be noted here that aspirin enhances the formation of LXA_4 as discussed above, suggesting that perhaps, enhanced formation of LXA_4 is responsible for the beneficial action observed.

It was reported[116] that increased PGE_2 produced in Kupffer cells attenuated insulin-dependent glucose utilization by interrupting the intracellular signal chain downstream of the insulin receptor in hepatocytes. In addition, PGE_2 stimulated **oncostatin M (OSM) production** by Kupffer cells that, in turn, attenuated insulin-dependent Akt activation and inhibited the expression of key enzymes of hepatic lipid metabolism. Since both COX-2 and OSM mRNA are induced early in the course of the development of NAFLD and NASH, it indicates that induction of OSM production in Kupffer cells by an autocrine PGE_2-dependent feed-forward loop may be an additional mechanism that contributes to hepatic insulin resistance and the development of NAFLD and NASH. The importance of activation of Kupffer cells in NASH and NAFLD lies in the fact that the metabolic abnormalities seen in these conditions in the form of insulin resistance and low-grade systemic inflammation could lead to enhanced release of free fatty acid flux and changes in adipocytokines production such as leptin, adiponectin and IL-6 as discussed above. As a result, the nuclear transcription factor peroxisome proliferator-activated receptor γ and the endocannabinoid system (that are also formed from AA) are activated that may predispose to the development of liver **fibrosis**[117,118]. Hence, early identification and management of NASH and NAFLD is important.

CONCLUSION

NAFLD is associated with decreased levels of AA, EPA and DHA and their anti-inflammatory products PGE_1, PGD_2, LXs, resolvins and protectins with a concomitant increase in pro-inflammatory cytokines IL-6 and TNF-α and bioactive lipids PGE_2, LTs and TXs. The low levels of AA, EPA and DHA could be a result of decreased activity of Δ^6 and Δ^5 desaturases. In view of this, administration of AA/EPA/DHA and/or more stable synthetic analogues of LXs, resolvins and protectins may prove to be useful in the prevention management and assessing prognosis of NAFLD and possibly other inflammatory diseases[91]. This proposal can be verified by estimating plasma, liver and adipose tissue content of AA, EPA, DHA, LXs, resolvins and protectins and the activity of Δ^6 and Δ^5 desaturases to the stage and activity of NASH and NAFLD. Periodic estimation of plasma, adipose and hepatic content of various PUFAs, LXs, resolvins and protectins and the activity of Δ^6 and Δ^5 desaturases and correlating them to the response to treatment is recommended. It is predicted that those in whom the plasma, adipose and hepatic content of various PUFAs, LXs, resolvins and protectins and the activity of Δ^6 and Δ^5 desaturases show an increase can be regarded as responding favorably to treatment while those in whom there is no change or decrease in the levels of these bioactive lipids and activity of Δ^6 and Δ^5 desaturases are likely to have progressive disease or unresponsive treatment that is being offered. Such patients need more aggressive therapy. Thus, plasma, adipose and hepatic content of various PUFAs, LXs, resolvins and protectins and the activity of Δ^6 and Δ^5 desaturases can be used to predict prognosis of NASH and NAFLD.

REFERENCES

1 **Meigs JB**. Invited commentary: insulin resistance syndrome? Syndrome X? Multiple metabolic syndrome? A syndrome at all? Factor analysis reveals patterns in the fabric of correlated metabolic risk factors. *Am J Epidemiol* 2000; **152**: 908-911; discussion 912

2 **Marchesini G**, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917-923

3 **Marchesini G**, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* 1999; **107**: 450-455

4 **Angelico F**, Del Ben M, Conti R, Francioso S, Feole K, Fiorello S, Cavallo MG, Zalunardo B, Lirussi F, Alessandri C, Violi F. Insulin resistance, the metabolic syndrome, and nonalcoholic fatty liver disease. *J Clin Endocrinol Metab* 2005; **90**: 1578-1582

5 **Adams LA**, Angulo P. Recent concepts in non-alcoholic fatty liver disease. *Diabet Med* 2005; **22**: 1129-1133

6 **Bayard M**, Holt J, Boroughs E. Nonalcoholic fatty liver disease. *Am Fam Physician* 2006; **73**: 1961-1968

7 **Crespo J**, Cayón A, Fernández-Gil P, Hernández-Guerra M, Mayorga M, Domínguez-Díez A, Fernández-Escalante JC, Pons-Romero F. Gene expression of tumor necrosis factor alpha and TNF-receptors, p55 and p75, in nonalcoholic steatohepatitis patients. *Hepatology* 2001; **34**: 1158-1163

8 **Bahcecioglu IH**, Yalniz M, Ataseven H, Ilhan N, Ozercan IH, Seckin D, Sahin K. Levels of serum hyaluronic acid, TNF-alpha and IL-8 in patients with nonalcoholic steatohepatitis. *Hepatogastroenterology* 2005; **52**: 1549-1553

9 **Mas E**, Danjoux M, Garcia V, Carpentier S, Ségui B, Levade T. IL-6 deficiency attenuates murine diet-induced non-alcoholic steatohepatitis. *PLoS One* 2009; **4**: e7929

10 **Memon RA**, Grunfeld C, Feingold KR. TNF-alpha is not the cause of fatty liver disease in obese diabetic mice. *Nat Med* 2001; **7**: 2-3

11 **Deng QG**, She H, Cheng JH, French SW, Koop DR, Xiong S, Tsukamoto H. Steatohepatitis induced by intragastric overfeeding in mice. *Hepatology* 2005; **42**: 905-914

12 **Ii H**, Hatakeyama S, Tsutsumi K, Sato T, Akiba S. Group IVA phospholipase A2 is associated with the storage of lipids in adipose tissue and liver. *Prostaglandins Other Lipid Mediat* 2008; **86**: 12-17

13 **Pérez S**, Aspichueta P, Ochoa B, Chico Y. The 2-series prostaglandins suppress VLDL secretion in an inflammatory condition-dependent manner in primary rat hepatocytes. *Biochim Biophys Acta* 2006; **1761**: 160-171

14 **Enomoto N**, Ikejima K, Yamashina S, Enomoto A, Nishiura T, Nishimura T, Brenner DA, Schemmer P, Bradford BU, Rivera CA, Zhong Z, Thurman RG. Kupffer cell-derived prostaglandin E(2) is involved in alcohol-induced fat accumulation in rat liver. *Am J Physiol Gastrointest Liver Physiol* 2000; **279**: G100-G106

15 **Ii H**, Yokoyama N, Yoshida S, Tsutsumi K, Hatakeyama S, Sato T, Ishihara K, Akiba S. Alleviation of high-fat diet-induced fatty liver damage in group IVA phospholipase A2-knockout mice. *PLoS One* 2009; **4**: e8089

16 **Carmiel-Haggai M**, Cederbaum AI, Nieto N. A high-fat diet leads to the progression of non-alcoholic fatty liver disease in obese rats. *FASEB J* 2005; **19**: 136-138

17 **Das UN**. Lipoxins, resolvins, protectins, maresins, and nitrolipids: Connecting lipids, inflammation, and cardiovascular disease risk. *Curr Cardiovasc Risk Rep* 2010; **4**: 24-31

18 **Das UN**. Essential fatty acids and their metabolites could function as endogenous HMG-CoA reductase and ACE enzyme inhibitors, anti-arrhythmic, anti-hypertensive, anti-atherosclerotic, anti-inflammatory, cytoprotective, and cardioprotective molecules. *Lipids Health Dis* 2008; **7**: 37

19 **Machado FS**, Johndrow JE, Esper L, Dias A, Bafica A, Serhan CN, Aliberti J. Anti-inflammatory actions of lipoxin A4 and aspirin-triggered lipoxin are SOCS-2 dependent. *Nat Med* 2006; **12**: 330-334

20 **Alwayn IP**, Gura K, Nosé V, Zausche B, Javid P, Garza J, Verbesey J, Voss S, Ollero M, Andersson C, Bistrian B, Folkman J, Puder M. Omega-3 fatty acid supplementation prevents hepatic steatosis in a murine model of nonalcoholic fatty liver disease. *Pediatr Res* 2005; **57**: 445-452

21 **Araya J**, Rodrigo R, Videla LA, Thielemann L, Orellana M, Pettinelli P, Poniachik J. Increase in long-chain polyunsaturated fatty acid n - 6/n - 3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease. *Clin Sci* (Lond) 2004; **106**: 635-643

22 **Das UN**. Clinical laboratory tools to diagnose inflammation. *Adv Clin Chem* 2006; **41**: 189-229

23 **Serhan CN**. Lipoxins and aspirin-triggered 15-epi-lipoxins are the first lipid mediators of endogenous anti-inflammation and resolution. *Prostaglandins Leukot Essent Fatty Acids* 2005; **73**: 141-162

24 **Clària J**, Serhan CN. Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. *Proc Natl Acad Sci USA* 1995; **92**: 9475-9479

25 **Das UN**. Essential fatty acids: biochemistry, physiology and pathology. *Biotechnol J* 2006; **1**: 420-439

26 **Serhan CN**, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL. Resolvins: a family of bioactive

products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J Exp Med* 2002; **196**: 1025-1037
27 **Das UN**. Essential Fatty acids - a review. *Curr Pharm Biotechnol* 2006; **7**: 467-482
28 **Chiang N**, Arita M, Serhan CN. **Anti-inflammatory circuitry**: lipoxin, aspirin-triggered lipoxins and their receptor ALX. *Prostaglandins Leukot Essent Fatty Acids* 2005; **73**: 163-177
29 **Levy BD**, Clish CB, Schmidt B, Gronert K, Serhan CN. Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol* 2001; **2**: 612-619
30 **Serhan CN**, Maddox JF, Petasis NA, Akritopoulou-Zanze I, Papayianni A, Brady HR, Colgan SP, Madara JL. Design of lipoxin A4 stable analogs that block transmigration and adhesion of human neutrophils. *Biochemistry* 1995; **34**: 14609-14615
31 **Das UN**. Current and emerging strategies for the treatment and management of systemic lupus erythematosus based on molecular signatures of acute and chronic inflammation. *J Inflamm Res* 2010; **3**: 143-170
32 **Das UN**. Molecular basis of health and disease. New York: Springer, 2011
33 **Zordoky BN**, El-Kadi AO. Effect of cytochrome P450 polymorphism on arachidonic acid metabolism and their impact on cardiovascular diseases. *Pharmacol Ther* 2010; **125**: 446-463
34 **Nithipatikom K**, Gross GJ. Review article: epoxyeicosatrienoic acids: novel mediators of cardioprotection. *J Cardiovasc Pharmacol Ther* 2010; **15**: 112-119
35 **Campbell WB**, Fleming I. Epoxyeicosatrienoic acids and endothelium-dependent responses. *Pflugers Arch* 2010; **459**: 881-895
36 **Arnold C**, Konkel A, Fischer R, Schunck WH. Cytochrome P450-dependent metabolism of omega-6 and omega-3 long-chain polyunsaturated fatty acids. *Pharmacol Rep* 2010; **62**: 536-547
37 **Kroetz DL**, Zeldin DC. Cytochrome P450 pathways of arachidonic acid metabolism. *Curr Opin Lipidol* 2002; **13**: 273-283
38 **Gao L**, Yin H, Milne GL, Porter NA, Morrow JD. **Formation** of F-ring isoprostane-like compounds (F3-isoprostanes) in vivo from eicosapentaenoic acid. *J Biol Chem* 2006; **281**: 14092-14099
39 **Serhan CN**, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. *J Exp Med* 2000; **192**: 1197-1204
40 **Serhan CN**, Clish CB, Brannon J, Colgan SP, Gronert K, Chiang N. Anti-microinflammatory lipid signals generated from dietary N-3 fatty acids via cyclooxygenase-2 and transcellular processing: a novel mechanism for NSAID and N-3 PUFA therapeutic actions. *J Physiol Pharmacol* 2000; **51**: 643-654
41 **Marcheselli VL**, Hong S, Lukiw WJ, Tian XH, Gronert K, Musto A, Hardy M, Gimenez JM, Chiang N, Serhan CN, Bazan NG. Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and pro-inflammatory gene expression. *J Biol Chem* 2003; **278**: 43807-43817
42 **Hong S**, Gronert K, Devchand PR, Moussignac RL, Serhan CN. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. *J Biol Chem* 2003; **278**: 14677-14687
43 **Serhan CN**. Novel lipid mediators and resolution mechanisms in acute inflammation: to resolve or not? *Am J Pathol* 2010; **177**: 1576-1591
44 **Masoodi M**, Mir AA, Petasis NA, Serhan CN, Nicolaou A. Simultaneous lipidomic analysis of three families of bioactive lipid mediators leukotrienes, resolvins, protectins and related hydroxy-fatty acids by liquid chromatography/electrospray ionisation tandem mass spectrometry. *Rapid Commun Mass Spectrom* 2008; **22**: 75-83
45 **Romano M**, Luciotti G, Gangemi S, Marinucci F, Prontera C, D'Urbano E, Davì G. Urinary excretion of lipoxin A(4) and related compounds: development of new extraction techniques for lipoxins. *Lab Invest* 2002; **82**: 1253-1254
46 **Gangemi S**, Luciotti G, D'Urbano E, Mallamace A, Santoro D, Bellinghieri G, Davi G, Romano M. Physical exercise increases urinary excretion of lipoxin A4 and related compounds. *J Appl Physiol* 2003; **94**: 2237-2240
47 **Gangemi S**, Pescara L, D'Urbano E, Basile G, Nicita-Mauro V, Davì G, Romano M. Aging is characterized by a profound reduction in anti-inflammatory lipoxin A4 levels. *Exp Gerontol* 2005; **40**: 612-614
48 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231
49 **Clark JM**, Brancati FL, Diehl AM. Nonalcoholic fatty liver disease. *Gastroenterology* 2002; **122**: 1649-1657
50 **Clark JM**, Diehl AM. Defining nonalcoholic fatty liver disease: implications for epidemiologic studies. *Gastroenterology* 2003; **124**: 248-250
51 **Teli MR**, James OF, Burt AD, Bennett MK, Day CP. The natural history of nonalcoholic fatty liver: a follow-up study. *Hepatology* 1995; **22**: 1714-1719
52 **Clark JM**, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol* 2003; **98**: 960-967
53 **Bellentani S**, Saccoccio G, Masutti F, Crocè LS, Brandi G, Sasso F, Cristanini G, Tiribelli C. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000; **132**: 112-117
54 **Wanless IR**, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology* 1990; **12**: 1106-1110
55 **Clark JM**, Diehl AM. Hepatic steatosis and type 2 diabetes mellitus. *Curr Diab Rep* 2002; **2**: 210-215
56 **Sahoo S**, Hart J. Histopathological features of L-asparaginase-induced liver disease. *Semin Liver Dis* 2003; **23**: 295-299
57 **Sax HC**, Talamini MA, Brackett K, Fischer JE. **Hepatic steatosis** in total parenteral nutrition: failure of fatty infiltration to correlate with abnormal serum hepatic enzyme levels. *Surgery* 1986; **100**: 697-704
58 **Sax HC**, Bower RH. Hepatic complications of total parenteral nutrition. *JPEN J Parenter Enteral Nutr* 1988; **12**: 615-618
59 **Benjamin DR**. Hepatobiliary dysfunction in infants and children associated with long-term total parenteral nutrition. A clinico-pathologic study. *Am J Clin Pathol* 1981; **76**: 276-283
60 **Van Aerde JE**, Duerksen DR, Gramlich L, Meddings JB, Chan G, Thomson AB, Clandinin MT. Intravenous fish oil emulsion attenuates total parenteral nutrition-induced cholestasis in newborn piglets. *Pediatr Res* 1999; **45**: 202-208
61 **Yeh SL**, Chang KY, Huang PC, Chen WJ. Effects of n-3 and n-6 fatty acids on plasma eicosanoids and liver antioxidant enzymes in rats receiving total parenteral nutrition. *Nutrition* 1997; **13**: 32-36
62 **Chen WJ**, Yeh SL. Effects of fish oil in parenteral nutrition. *Nutrition* 2003; **19**: 275-279
63 **Sekiya M**, Yahagi N, Matsuzaka T, Najima Y, Nakakuki M, Nagai R, Ishibashi S, Osuga J, Yamada N, Shimano H. Polyunsaturated fatty acids ameliorate hepatic steatosis in obese mice by SREBP-1 suppression. *Hepatology* 2003; **38**: 1529-1539
64 **Das UN**. Metabolic syndrome pathophysiology: the role of essential fatty acids. Ames: Wiley-Blackwell, 2010
65 **Diehl AM**, Li ZP, Lin HZ, Yang SQ. Cytokines and the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2005; **54**: 303-306
66 **Das UN**. Prostaglandins and immuneresponse in cancer. *Int J Tiss Reac* 1980; **2**: 233-236
67 **Das UN**. Auto-immunity and prostaglandins. *Int J Tissue React* 1981; **3**: 89-94

68 **Das UN**. Inhibition of sensitized lymphocyte response to sperm antigen(s) by prostaglandins. *IRCS Med Sci* 1981; **9**: 1087

69 **Sravan Kumar G**, Das UN, Vijay Kumar K, Madhavi N, Das NP, Tan BKH. Effect of n-6 and n-3 fatty acids on the proliferation of human lymphocytes and their secretion of TNF-α and IL-2 in vitro. *Nutr Res* 1992; **12**: 815-823

70 **Kumar GS**, Das UN. Effect of prostaglandins and their precursors on the proliferation of human lymphocytes and their secretion of tumor necrosis factor and various interleukins. *Prostaglandins Leukot Essent Fatty Acids* 1994; **50**: 331-334

71 **Madhavi N**, Das UN, Prabha PS, Kumar GS, Koratkar R, Sagar PS. **Suppression of human T-cell growth in vitro by cis-unsaturated fatty acids: relationship to free radicals and lipid peroxidation.** *Prostaglandins Leukot Essent Fatty Acids* 1994; **51**: 33-40

72 **Das UN**. Beneficial effect of eicosapentaenoic and docosahexaenoic acids in the management of systemic lupus erythematosus and its relationship to the cytokine network. *Prostaglandins Leukot Essent Fatty Acids* 1994; **51**: 207-213

73 **Das UN**. Oxidants, anti-oxidants, essential fatty acids, eicosanoids, cytokines, gene/oncogene expression and apoptosis in systemic lupus erythematosus. *J Assoc Physicians India* 1998; **46**: 630-634

74 **Li J**, Zhang H, Wu F, Nan Y, Ma H, Guo W, Wang H, Ren J, Das UN, Gao F. Insulin inhibits tumor necrosis factor-alpha induction in myocardial ischemia/reperfusion: role of Akt and endothelial nitric oxide synthase phosphorylation. *Crit Care Med* 2008; **36**: 1551-1558

75 **Das UN**. Is insulin an antiinflammatory molecule? *Nutrition* 2001; **17**: 409-413

76 **Das UN**. Can glucose-insulin-potassium regimen suppress inflammatory bowel disease? *Med Hypotheses* 2001; **57**: 183-185

77 **Das UN**. Hypothesis: can glucose-insulin-potassium regimen in combination with polyunsaturated fatty acids suppress lupus and other inflammatory conditions? *Prostaglandins Leukot Essent Fatty Acids* 2001; **65**: 109-113

78 **Das UN**. **The lipids that matter from infant nutrition to insulin resistance.** *Prostaglandins Leukot Essent Fatty Acids* 2002; **67**: 1-12

79 **Teixeira de Lemos E**, Reis F, Baptista S, Pinto R, Sepodes B, Vala H, Rocha-Pereira P, Correia da Silva G, Teixeira N, Silva AS, Carvalho L, Teixeira F, Das UN. **Exercise training** decreases proinflammatory profile in Zucker diabetic (type 2) fatty rats. *Nutrition* 2009; **25**: 330-339

80 **Das UN**. Exercise and inflammation. *Eur Heart J* 2006; **27**: 1385; author reply 1385-1386

81 **Das UN**. Anti-inflammatory nature of exercise. *Nutrition* 2004; **20**: 323-326

82 **Bonventre JV**. Phospholipase A2 and signal transduction. *J Am Soc Nephrol* 1992; **3**: 128-150

83 **Serhan CN**. Systems approach with inflammatory exudates uncovers novel anti-inflammatory and pro-resolving mediators. *Prostaglandins Leukot Essent Fatty Acids* 2008; **79**: 157-163

84 **Godson C**, Mitchell S, Harvey K, Petasis NA, Hogg N, Brady HR. Cutting edge: lipoxins rapidly stimulate nonphlogistic phagocytosis of apoptotic neutrophils by monocyte-derived macrophages. *J Immunol* 2000; **164**: 1663-1667

85 **Gilroy DW**, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA. Inducible cyclooxygenase may have anti-inflammatory properties. *Nat Med* 1999; **5**: 698-701

86 **Gilroy DW**, Newson J, Sawmynaden P, Willoughby DA, Croxtall JD. A novel role for phospholipase A2 isoforms in the checkpoint control of acute inflammation. *FASEB J* 2004; **18**: 489-498

87 **Cominelli F**, Nast CC, Llerena R, Dinarello CA, Zipser RD. Interleukin 1 suppresses inflammation in rabbit colitis. Mediation by endogenous prostaglandins. *J Clin Invest* 1990; **85**: 582-586

88 **Schwab JH**, Anderle SK, Brown RR, Dalldorf FG, Thompson RC. Pro- and anti-inflammatory roles of interleukin-1 in recurrence of bacterial cell wall-induced arthritis in rats. *Infect Immun* 1991; **59**: 4436-4442

89 **Serhan CN**, Petasis NA. Resolvins and protectins in inflammation resolution. *Chem Rev* 2011; **111**: 5922-5943

90 **Croxtall JD**, Choudhury Q, Tokumoto H, Flower RJ. Lipocortin-1 and the control of arachidonic acid release in cell signalling. Glucocorticoids (changed from glucorticoids) inhibit G protein-dependent activation of cPLA2 activity. *Biochem Pharmacol* 1995; **50**: 465-474

91 **Das UN**. Lipoxins as biomarkers of lupus and other inflammatory conditions. *Lipids Health Dis* 2011; **10**: 76

92 **Brenner RR**. **Hormonal modulation of delta6 and delta5 desaturases: case of diabetes.** *Prostaglandins Leukot Essent Fatty Acids* 2003; **68**: 151-162

93 **Hatzitolios A**, Savopoulos C, Lazaraki G, Sidiropoulos I, Haritanti P, Lefkopoulos A, Karagiannopoulou G, Tzioufa V, Dimitrios K. Efficacy of omega-3 fatty acids, atorvastatin and orlistat in non-alcoholic fatty liver disease with dyslipidemia. *Indian J Gastroenterol* 2004; **23**: 131-134

94 **Puri P**, Baillie RA, Wiest MM, Mirshahi F, Choudhury J, Cheung O, Sargeant C, Contos MJ, Sanyal AJ. A lipidomic analysis of nonalcoholic fatty liver disease. *Hepatology* 2007; **46**: 1081-1090

95 **Kühn H**, O'Donnell VB. Inflammation and immune regulation by 12/15-lipoxygenases. *Prog Lipid Res* 2006; **45**: 334-356

96 **Chen M**, Yang ZD, Smith KM, Carter JD, Nadler JL. Activation of 12-lipoxygenase in proinflammatory cytokine-mediated beta cell toxicity. *Diabetologia* 2005; **48**: 486-495

97 **Chakrabarti SK**, Cole BK, Wen Y, Keller SR, Nadler JL. 12/15-lipoxygenase products induce inflammation and impair insulin signaling in 3T3-L1 adipocytes. *Obesity* (Silver Spring) 2009; **17**: 1657-1663

98 **Das UN**. Obesity, metabolic syndrome X, and inflammation. *Nutrition* 2002; **18**: 430-432

99 **Das UN**. Is metabolic syndrome X an inflammatory condition? *Exp Biol Med* (Maywood) 2002; **227**: 989-997

100 **Das UN**. Pathobiology of metabolic syndrome X in obese and non-obese South Asian Indians: further discussion and some suggestions. *Nutrition* 2003; **19**: 560-562

101 **Das UN**. Metabolic syndrome X is common in Indians: but, why and how? *J Assoc Physicians India* 2003; **51**: 987-998

102 **Das UN**. Metabolic syndrome X: an inflammatory condition? *Curr Hypertens Rep* 2004; **6**: 66-73

103 **Das UN**. Metabolic syndrome X is a low-grade systemic inflammatory condition with its origins in the perinatal period. *Curr Nutr Food Sci* 2007; **3**: 277-295

104 **Das UN**. Metabolic syndrome is a low-grade systemic inflammatory condition. *Expert Rev Endocrinol Metab* 2010; **5**: 577-592

105 **Cyrus T**, Witztum JL, Rader DJ, Tangirala R, Fazio S, Linton MF, Funk CD. Disruption of the 12/15-lipoxygenase gene diminishes atherosclerosis in apo E-deficient mice. *J Clin Invest* 1999; **103**: 1597-1604

106 **Cyrus T**, Praticò D, Zhao L, Witztum JL, Rader DJ, Rokach J, FitzGerald GA, Funk CD. Absence of 12/15-lipoxygenase expression decreases lipid peroxidation and atherogenesis in apolipoprotein e-deficient mice. *Circulation* 2001; **103**: 2277-2282

107 **Bleich D**, Chen S, Zipser B, Sun D, Funk CD, Nadler JL. Resistance to type 1 diabetes induction in 12-lipoxygenase knockout mice. *J Clin Invest* 1999; **103**: 1431-1436

108 **McDuffie M**, Maybee NA, Keller SR, Stevens BK, Garmey JC, Morris MA, Kropf E, Rival C, Ma K, Carter JD, Tersey SA, Nunemaker CS, Nadler JL. Nonobese diabetic (NOD) mice congenic for a targeted deletion of 12/15-lipoxygenase are protected from autoimmune diabetes. *Diabetes* 2008; **57**: 199-208

109 **Nunemaker CS**, Chen M, Pei H, Kimble SD, Keller SR, Carter JD, Yang Z, Smith KM, Wu R, Bevard MH, Garmey JC, Nadler JL. 12-Lipoxygenase-knockout mice are resistant to

inflammatory effects of obesity induced by Western diet. *Am J Physiol Endocrinol Metab* 2008; **295**: E1065-E1075
110 **Sears DD**, Miles PD, Chapman J, Ofrecio JM, Almazan F, Thapar D, Miller YI. 12/15-lipoxygenase is required for the early onset of high fat diet-induced adipose tissue inflammation and insulin resistance in mice. *PLoS One* 2009; **4**: e7250
111 **Kayama Y**, Minamino T, Toko H, Sakamoto M, Shimizu I, Takahashi H, Okada S, Tateno K, Moriya J, Yokoyama M, Nojima A, Yoshimura M, Egashira K, Aburatani H, Komuro I. Cardiac 12/15 lipoxygenase-induced inflammation is involved in heart failure. *J Exp Med* 2009; **206**: 1565-1574
112 **González-Périz A**, Horrillo R, Ferré N, Gronert K, Dong B, Morán-Salvador E, Titos E, Martínez-Clemente M, López-Parra M, Arroyo V, Clària J. Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: a role for resolvins and protectins. *FASEB J* 2009; **23**: 1946-1957
113 **Araya J**, Rodrigo R, Pettinelli P, Araya AV, Poniachik J, Videla LA. Decreased liver fatty acid delta-6 and delta-5 desaturase activity in obese patients. *Obesity* (Silver Spring) 2010; **18**: 1460-1463
114 **Park H**, Shima T, Yamaguchi K, Mitsuyoshi H, Minami M, Yasui K, Itoh Y, Yoshikawa T, Fukui M, Hasegawa G, Nakamura N, Ohta M, Obayashi H, Okanoue T. Efficacy of long-term ezetimibe therapy in patients with nonalcoholic fatty liver disease. *J Gastroenterol* 2011; **46**: 101-107
115 **Fujita K**, Nozaki Y, Wada K, Yoneda M, Endo H, Takahashi H, Iwasaki T, Inamori M, Abe Y, Kobayashi N, Kirikoshi H, Kubota K, Saito S, Nagashima Y, Nakajima A. Effectiveness of antiplatelet drugs against experimental non-alcoholic fatty liver disease. *Gut* 2008; **57**: 1583-1591
116 **Henkel J**, Gärtner D, Dorn C, Hellerbrand C, Schanze N, Elz SR, Püschel GP. Oncostatin M produced in Kupffer cells in response to PGE2: possible contributor to hepatic insulin resistance and steatosis. *Lab Invest* 2011; **91**: 1107-1117
117 **Anty R**, Lemoine M. Liver fibrogenesis and metabolic factors. *Clin Res Hepatol Gastroenterol* 2011; **35** Suppl 1: S10-S20
118 **Tilg H**. Adipocytokines in nonalcoholic fatty liver disease: key players regulating steatosis, inflammation and fibrosis. *Curr Pharm Des* 2010; **16**: 1893-1895
119 **Serhan CN**, Chiang N. Lipid-derived mediators in endogenous anti-inflammation and resolution: lipoxins and aspirin-triggered 15-epi-lipoxins. *ScientificWorldJournal* 2002; **2**: 169-204

S- Editor Wu X **L- Editor** Roemmele A **E- Editor** Zheng XM

World Journal of Diabetes

Online Submissions: http://www.wjgnet.com/1948-9358office
wjd@wjgnet.com
doi:10.4239/wjd.v2.i11.189

World J Diabetes 2011 November 15; 2(11): 189-195
ISSN 1948-9358 (online)

EDITORIAL

Disordered eating behaviors in type 1 diabetic patients

Alejandra Larrañaga, María F Docet, Ricardo V García-Mayor

Alejandra Larrañaga, Ricardo V García-Mayor, Eating Disorders Unit, University Hospital of Vigo, 36204 Vigo, Spain
María F Docet, Nutrition Section, University Hospital of Vigo, 36204 Vigo, Spain
Author contributions: Larrañaga A, Docet MF and García-Mayor RV contributed equally to this work.
Supported by a grant from INCITE, Consellería Innovación e Industria, Galician Government
Correspondence to: Ricardo V García-Mayor, Professor, Eating Disorders Unit, University Hospital of Vigo, PO Box 1691, 36204 Vigo, Spain. ricardo.garcia.mayor@sergas.es
Telephone: +34-986-816000 Fax: +34-986-816029
Received: August 27, 2011 Revised: October 5, 2011
Accepted: October 22, 2011
Published online: November 15, 2011

Abstract

Patients with type 1 diabetes mellitus are at high risk for disordered eating behaviors (DEB). Due to the fact that type 1 diabetes mellitus is one of the most common chronic illnesses of childhood and adolescence, the coexistence of eating disorders (ED) and diabetes often affects adolescents and young adults. Since weight management during this state of development can be especially difficult for those with type 1 diabetes, some diabetics may restrict or omit insulin, a condition known as diabulimia, as a form of weight control. It has been clearly shown that ED in type 1 diabetics are associated with impaired metabolic control, more frequent episodes of ketoacidosis and an earlier than expected onset of diabetes-related microvascular complications, particularly retinopathy. The management of these conditions requires a multidisciplinary team formed by an endocrinologist/diabetologist, a nurse educator, a nutritionist, a psychologist and, frequently, a psychiatrist. The treatment of type 1 diabetes patients with DEB and ED should have the following components: diabetes treatment, nutritional management and psychological therapy. A high index of suspicion of the presence of an eating disturbance, particularly among those patients with persistent poor metabolic control, repeated episodes of ketoacidosis and/or weight and shape concerns are recommended in the initial stage of diabetes treatment, especially in young women. Given the extent of the problem and the severe medical risk associated with it, more clinical and technological research aimed to improve its treatment is critical to the future health of this at-risk population.

Key words: Anorexia nervosa; Bulimia nervosa; Eating disorders not otherwise specified; Disordered eating behaviors; Type 1 diabetes

Peer reviewers: Motoaki Saito, Dr., Department of Mol Pharmacology, Tottori University, 86 Nishimachi, Yonago 683-8503, Japan; Pappachan M Joseph, Dr., Department of Medicine, Pariyaram Medical College, C/o Adv Nicholas Joseph, Court Road, Taliparamba, Kannur 670141, India; Rob Weijers, Dr., Teaching Hospital, Onze Lieve Vrouwe Gasthuis, Oosterpark 9, Amsterdam, 1090 HM, The Netherlands

Larrañaga A, Docet MF, García-Mayor RV. Disordered eating behaviors in type 1 diabetic patients. *World J Diabetes* 2011; 2(11): 189-195 Available from: URL: http://www.wjgnet.com/1948-9358/full/v2/i11/189.htm DOI: http://dx.doi.org/10.4239/wjd.v2.i11.189

INTRODUCTION

Diabetes mellitus has been found to be the sixth leading cause of death for those living in the United States, affecting the young and old people at an alarming rate[1]. Type 1 diabetes typically has an early onset in life but can occur at any age. As the majority of patients with type 1 diabetes are children or adolescents, the nutritional anomalies have important consequences. To be diagnosed with diabetes represents a very hard experience that requires subsequent psychological adaptation. Unfortunately, this often does not occur and is followed by frustration and the non-acceptance of the disease.

The management of diabetes and its associated health-

risk factors are often complex and require considerable patient education and frequent medical monitoring[2]. The participation of patients is basic in order to obtain a correct degree of metabolic control; however, this carries a considerable amount of stress as a consequence. People on insulin must learn how to regulate their blood sugars by monitoring blood glucose levels daily while carefully attending to their food intake and an exercise regimen. Careful blood glucose monitoring is necessary to prevent wide variations in blood sugars that affect both short and long term health and functioning. Hypoglycemia reactions are a concern in the short run not only because they are frightening and disruptive, but also because, when severe, they can lead to unconsciousness, coma and death[3].

The three diagnostic forms of eating disorders (ED) are Anorexia Nervosa, Bulimia Nervosa and Eating Disorder Not Otherwise Specified. Common to all three is a core problem in which self-evaluation is unduly influenced by body weight or shape. According to the Diagnostic and Statistical Manual of Mental Disorders 4th Ed[4], Anorexia nervosa is an eating disorder characterized by refusal to maintain a healthy body weight and an obsessive fear of gaining weight. It is often coupled with a distorted self image which may be maintained by various cognitive biases that alter how the affected individual evaluates and thinks about her or his body, food and eating. Bulimia nervosa is characterized by binge eating, or consuming a large amount of food in a short amount of time, followed by an attempt to rid oneself of the calories consumed, usually by purging (vomiting) and/or by laxatives, diuretics or excessive exercise. ED Not Otherwise Specified are described as disorders of eating that do not meet the criteria for any specific eating disorder.

Type 1 diabetic patients have a high risk of suffering from ED as these patients have to select the food they eat carefully in an early period of their development and because both entities, type 1 diabetes and ED, often affect adolescents and young adults. Furthermore, adolescents with type 1 diabetes also suffer from other anomalies related to their eating behavior since they commonly diet or exercise to control weight and to overcome body dissatisfaction. Other adolescents, mainly girls, may present more severe misbehaviors such as splitting insulin doses or restricting food intake in order to reduce their body weight, bingeing and purging, using laxatives or adhering to an overly strict exercise regimen. All the above mentioned misbehaviors are called disordered eating behaviors (DEB)[5,6].

In this study, we will review these psychological anomalies suffered by type 1 diabetic patients, especially DEB and ED, and also discuss some aspects of their forms of presentation, management and prevention.

EPIDEMIOLOGY OF DISORDERED EATING BEHAVIOR AND ED IN TYPE 1 DIABETIC PATIENTS

Disordered eating behavior is common in young women living in westernized countries where thinness is valued and dietary restraint is pursued[7]. Prevalence studies in North America indicate that full syndrome bulimia nervosa may be found in 1%-3% of adolescents and young adult women and subthreshold disorders are even more common[8,9]. The rates of these disorders are lower but rising in less-westernized countries such as in Asia and Africa as Western attitudes towards weight and shape become more pervasive[10-12]. However, survey findings indicate that the number of subjects with ED or abnormal eating attitudes has increased significantly during the past three decades in non-Western countries.

In Japan, the rate of ED ranges from 0.025% to 0.2% for Anorexia Nervosa and from 1.9% to 2.9% for Bulimia Nervosa[13]. Nasser[14] in Cairo reported that the estimated prevalence of Bulimia Nervosa was 1.2% among the school girls and, using the same type of survey as the one used in Cairo, investigators estimated that 3.2% of Iranian school girls suffer from Bulimia Nervosa. Buhrich[15] reported that 0.05% of the psychiatric patient samples in Malaysia were diagnosed with Anorexia Nervosa and this prevalence rate had not increased for 15 years. Differences in the prevalence of ED varies according to different ethnic groups[16,17]. However, a study found that ethnic differences in eating disorder symptoms disappeared when body mass index was controlled[18]. At present, there is no information on the effect of culture and race on ED in people with diabetes.

The risk of eating disturbances has been postulated to be higher in type 1 diabetic patients than in the general population due to multiple interacting factors related to diabetes and its treatment[19,20]. Diabetes management imposes some degree of perceived dietary restraint, particularly patients who eat according to a predetermined meal plan rather than in response to internal cues for hunger and satiety. Such neglect of internal cues may contribute to dietary dysregulation in susceptible individuals[21]. The relationship between higher weight and disordered eating behavior presents a management dilemma for clinicians, since both dietary restraint and higher weight are clear risk factors for the development of ED and their negative health consequences.

BEHAVIORAL ANOMALIES ASSOCIATED WITH BODY WEIGHT OR SHAPE DISSATISFACTION IN TYPE 1 DIABETICS

The association of chronic illness, such as type 1 diabetes, asthma, attention deficit disorder, physical disabilities and seizure disorders, with disordered eating behavior is well known[5,6].

By controlling diabetes with insulin injections, many diabetics face a constant struggle with their weight[22]. As insulin encourages fat storage, many people with type 1 diabetes have discovered the relationship between reducing the amount of insulin they take and their corresponding weight loss[23]. It is well-known that adolescents with type 1 diabetes tend to exhibit increased difficulty in

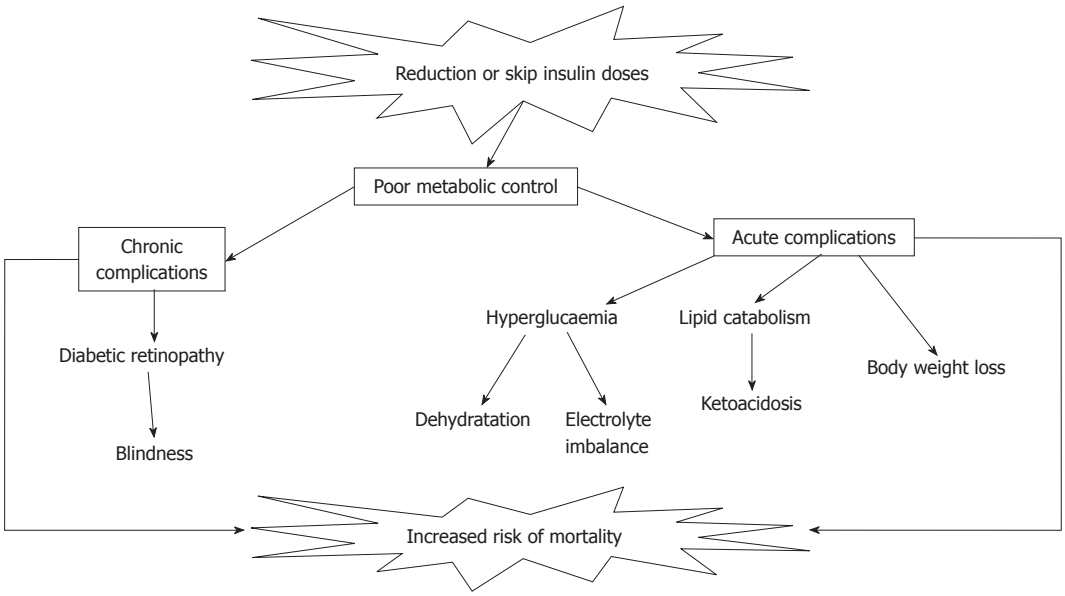


Figure 1 Consequences of reducing or skipping insulin doses in type 1 diabetes patients.

maintaining optimal weight and also are more inclined to be concerned about their weight than their non-diabetic counterparts[24].

Since weight management during this state of development can be especially difficult for those with type 1 diabetes, some diabetics may restrict or omit insulin, a condition known as diabulimia, as a form of weight control[25-27]. In a study that looked at 143 adolescents with type 1 diabetes who completed the Assessing Health and Eating among Adolescents with Diabetes survey, unhealthy weight control practice was observed in 37.9% of females and 15.9% of males. Among the females, 10.3% reported skipping insulin and 7.4% reported taking less insulin to control their weight[28]. Only one male reported doing either of these behaviors. In another 4-year follow-up study of 91 girls with diabetes aged 12 to 18, dieting was reported by 38% of the sample, binge eating by 45%, insulin omission by 14% and self-induced vomiting by 8% at baseline. These behaviors were even more common at follow-up, when most of the girls were at the age of the highest risk for ED. At this time, more than half of the sample reported dieting for weight loss and binge eating and one-third reported deliberate insulin omission to prevent weight gain[29].

In general terms, it is estimated that between 30% and 40% of adolescents and young adults with diabetes skip insulin after meals to lose weight[30].

CONSEQUENCES OF DISORDERED EATING BEHAVIORS IN TYPE 1 DIABETICS

A spectrum of severity of disturbance of eating habits and attitudes and subthreshold eating problems, seen as relatively mild in non diabetic patients, can give rise to clinically important disturbances of self-care and glycemia control in diabetics. In general terms, glycosilated hemoglobin was higher in patients with diabetes who had ED compared with those with diabetes without ED[29-31].

The lack of proper insulin treatment in type 1 diabetics may lead to many harmful physical effects. Reducing insulin to lose weight increases the risk of dehydration, break down of muscle tissue and high risk of developing infections and fatigue. If this behavior continues, it may also result in kidney failure, eye disease leading to blindness[32,33], vascular disease and even death[34].

ED in type 1 diabetics have been clearly shown to be associated with impaired metabolic control[29,35-39], more frequent episodes of ketoacidosis[40] and an earlier than expected onset of diabetes-related microvascular complications, particularly retinopathy[29,38,41-43]. Furthermore, disordered eating status was more predictive of diabetic retinopathy than the duration of diabetes, which is a well-established risk factor for microvascular complications[44]. Furthermore, ED in type 1 diabetic patients is associated with high mortality[45].

Regarding mortality, an 11-year follow-up study reports that insulin restriction conveyed more than a three-fold increased risk of mortality in type 1 diabetic patients after controlling for age, body mass index and HbA1c values. Age of death was younger among insulin restrictors, with a mean age of death of 45 years, as compared to 58 years among those reporting appropriate insulin use[46] (Figure 1).

MANAGEMENT OF EATING BEHAVIORAL ANOMALIES IN TYPE 1 DIABETICS

Despite the fact that little research has been done to determine the best treatment approaches for the problem of type 1 diabetic patients with ED or disordered eating behavior, a multidisciplinary care team is considered the standard to treat these people. Such a team should

include an endocrinologist/diabetologist, a nurse educator, a nutritionist with ED and/or diabetes training and a psychologist or social worker to provide weekly therapy. Depending on the severity of related psychiatric symptoms, such as depression and anxiety, a psychiatrist for psychopharmacological evaluation and treatment should also be consulted. Team members must be allowed to frequently and openly communicate with each other to maintain congruent treatment approaches, messages and goals.

The treatment of type 1 diabetes patients with disordered eating behavior and ED have the following components.

Diabetes treatment

The diabetes team has the important responsibility of monitoring insulin regimens and providing education about diabetes management and potential complications to patients and families[47]. The traditional approaches to poor blood glucose control involving a stricter and more intensive monitoring of the diabetic management may increase the risk for disordered eating[20]. For this reason, a less rigid approach is recommended in the insulin regimen and nutrition therapy such as the dose adjustment for normal eating protocol, which is a 5 d training course aimed to provide patients with type 1 diabetes the skills to fit diabetes into their lives rather than their lives into diabetes through daily insulin adjustment together with a flexible diet[48]. Lowering the amount of time spent on diabetes management during the day may help to lessen stress associated with the diabetes, which may in turn help alleviate disordered eating behavior. Krakoff[47] suggested that self-destructive insulin manipulation within the context of an ED may also be an indirect call for help, signaling the need for more parental/adult intervention in patient's physical and mental health.

Trento *et al*[49] suggest that offering a carbohydrate counting program within a group care management approach may help patients with type 1 diabetes acquire better self-efficacy and to restructure their cognitive and lifestyle potential.

Technological advances can also be used to address specific treatment issues seen in these patients. For example, the first challenge that most patients face is weight gain associated with insulin restart. Patients need to be taught to indentify insulin edema, which may make them feel fat, bloated and uncomfortable, as temporary water retention that is different from the development of fatty tissue. Special tools designed to measure water-related weight *vs* lean muscle mass *vs* fat mass could help patients tolerate the temporary weight gain related to edema[46]. Additionally, newer insulin analogs show evidence of improving weight profiles which could be of help[50,51].

Nutritional management

The dietician must balance the difficult tasks of providing diabetes education, ED education, writing meal plans and defining weight goals for patients and families[47,52]. The challenge presents when trying to balance the goal of slow weight gain and/or maintenance with diabetes meal planning. As the patient continues to increase calorie intake, insulin doses will need to be adjusted to match the amount of food eaten, avoiding hyperglycemia. Establishing a realistic goal of good blood glucose control is recommended instead of optimal blood glucose levels (that means levels between 70 and 130 mg/dL before meals and less than 180 two hours after starting a meal, with a glycated hemoglobin (A1C) level less than 7 percent[53]) as the body readjusts to refeeding and the patient begins to benefit from psychotherapy. Multiple daily injections regimens that use insulin to carbohydrate ratios provide greater flexibility with meal times and amounts of food but do require increased blood glucose monitoring and insulin injections. Such intense diabetes management may increase the potential for disordered eating as the child or adolescent must think constantly about the effects of food, insulin and exercise on his or her blood glucose levels. This may not be an ideal approach to diabetes meal planning during the treatment and recovery from the ED. As the individual's physical and psychological health improves, the incorporation of more flexible meal-planning strategies may be useful. Care professionals, including nutrition therapists and diabetes educators, should be sensitive to weight-related changes and concerns in youths with type 1 diabetes. It is important for all health care professionals to be aware that weight loss may be related to glycemia control.

Psychological therapy

Psychoeducation is a useful method to aid the patient to develop skills that will help him or her to cope with a chronic disease. Therefore, it can be helpful in type 1 diabetic patients who have difficulties accepting the disease.

Individual psychotherapy, group and family therapy are the most common ways to treat ED[54]. There are no studies showing the best psychotherapy modality for patients with type 1 diabetes and ED or disordered eating behavior. Some authors propose individual therapy to help patients to recover from ED and diabetes mismanagement[47]. Adolescents with type 1 diabetes often struggle with emotional issues related to having the illness and use an ED as a maladaptive coping mechanism. Individual therapy can help patients to develop more healthy coping strategies. Often families of patients with diabetes and ED have not adequately coped with the feelings of grief related to having a chronic illness in the family and thus they have not adequately supported the patient with diabetes. Dysfuntional family dynamics can exacerbate difficulties of adjusting to the illness and of resolving issues of grief and loss associated with the diagnosis. Family therapy is recommended to help the family to develop more functional ways of relating and in addressing issues of grief and loss that may be contributing to ED symptoms.

A psychiatric intervention is recommended to make a comprehensive mental health evaluation and to explore

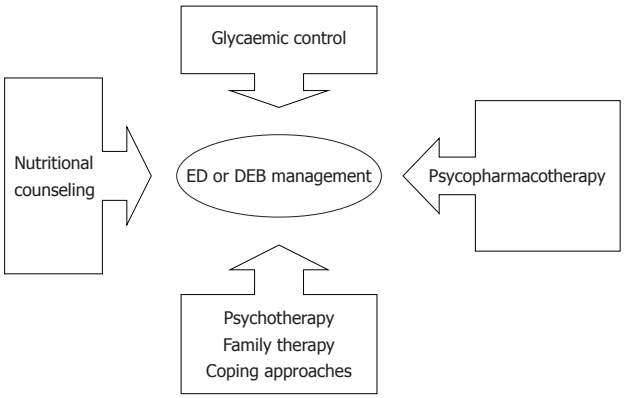


Figure 2 Components for eating disorders and disordered eating behaviors treatment in type 1 diabetes. DEB: Disordered eating behaviors; ED: Eating disorders.

Table 1 Prevention of eating disorders or disordered eating behaviors in type 1 diabetics

Avoid rigid glycaemic control in susceptible patients
High index of suspicion for health professional attended adolescents patients in order to precocious diagnosis
Consider psychological factors in patients with poor metabolic control
Use of a validate questionnaire in subjects with high risk of DEB or ED

ED: Eating disorders; DEB: Disordered eating behaviors.

the factors that might contribute to the eating disorder. The psychiatrist is also skilled in identifying and treating young people who may have other treatable problems such as depression, anxiety or substance abuse. Psychopharmacological agents such as fluoxetine and topiramate are useful to treat anxiety and binge eating episodes[52,55] (Figure 2).

PREVENTION

Since most type 1 diabetic patients do not admit to having an ED, this condition is commonly detected first by health care professionals[56]. The diabetes team may be the first to discover ED and can play a crucial role in recommending proper treatment to the patient and family. It is unlikely that diabetes management will improve until appropriate treatment begins for the concurrent ED.

Clinic-based group interventions for young women with diabetes and disordered eating behavior may be the most practical and nonstigmatizing approach to prevention and early intervention for this problem. Rigid approaches to the dietary management of diabetes can contribute to the development of disordered eating behavior. For these reasons, less intensive regimens are recommended in the initial stage of diabetes treatment, especially in young women[57-59].

It is recommended that the health care professionals who treat young women with type 1 diabetes maintain a high index of suspicion for the presence of an eating disturbance, particularly among those patients with persistent poor metabolic control, repeated episodes of ketoacidosis and/or weight and shape concerns[20,60].

Screening for DEB in type 1 diabetics would be the best approach for early detection of behavioral abnormalities in these patients; however, a validated screening tool is not available yet[60]. Recently, Markowitz *et al*[61] proposed a 16 item diabetes-specific self-reported measure of disordered eating as a brief screening tool for disordered eating in diabetes. The revised 16 item Diabetes Eating Problem Survey is a self-report measure of disordered eating that can be completed in less than 10 min. It has demonstrated excellent internal consistency, construct validity and external validity in a group of 112 youth with type 1 diabetes[61].

Individual or group interventions aimed to increase self-esteem, appearance and body acceptance and family-based interventions with the objective of developing flexible approaches to food and meal planning may help to avoid the development of disordered eating behavior in type 1 diabetic patients (Table 1).

CONCLUSION

Today it is well-known that DEB and subthreshold disordered ED are more prevalent in girls with type 1 diabetes than their peers without diabetes. Full established disordered eating behavior and ED are difficult to manage, requires a multidisciplinary team formed by an endocrinologist/diabetologist, nurse educator, nutritionist, psychologist and, frequently, a psychiatrist. The best psychological methods to treat these anomalies are not determined yet. Results of the treatment of these entities from experienced health professionals are not available yet. The key for the management is early diagnosis and treatment. Therefore, it is important that the staff of the diabetes team who treats these patients should know the relationship between poor diabetes metabolic control and intentional misuse of insulin or the recommended diet to control weight gain.

ED in type 1 diabetic patients represent some of the most complex patient problems to be treated both medically and psychologically. Given the extent of the problem and the severe medical risk associated with it, more clinical and technological research aimed to improve its treatment is critical to the future health of this at-risk population.

REFERENCES

1 **National Center for Health Statistics**, Centers for Disease Control and Prevention. National Diabetes Fact Sheet. USA Government. Hyattsville: University Town Center, 2011
2 **Koopmanschap M**. Coping with Type II diabetes: the patient's perspective. *Diabetologia* 2002; **45**: S18-S22
3 **Cox DJ**, Gonder-Frederick L. Major developments in behavioral diabetes research. *J Consult Clin Psychol* 1992; **60**: 628-638
4 **American Psychiatric Association**. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC: American Psychiatric Association, 1994

5 **Neumark-Sztainer D**, Story M, Falkner NH, Beuhring T, Resnick MD. Disordered eating among adolescents with chronic illness and disability: the role of family and other social factors. *Arch Pediatr Adolesc Med* 1998; **152**: 871-878
6 **Neumark-Sztainer D**, Story M, Resnick MD, Garwick A, Blum RW. Body dissatisfaction and unhealthy weight-control practices among adolescents with and without chronic illness: a population-based study. *Arch Pediatr Adolesc Med* 1995; **149**: 1330-1335
7 **Attie I**, Brooks-Gunn J. Development of eating problems in adolescent girls: A longitudinal study. *Dev Psychol* 1989; **25**: 70-79
8 **Fairburn CG**, Beglin SJ. Studies of the epidemiology of bulimia nervosa. *Am J Psychiatry* 1990; **147**: 401-408
9 **Jones JM**, Bennett S, Olmsted MP, Lawson ML, Rodin G. Disordered eating attitudes and behaviours in teenaged girls: a school-based study. *CMAJ* 2001; **165**: 547-552
10 **Hoek HW**. Review of the epidemiological studies of eating disorders. *Int Rev Psychiatry* 1993; **5**: 61-74
11 **Lee S**. How abnormal is the desire for slimness? A survey of eating attitudes and behaviour among Chinese undergraduates in Hong Kong. *Psychol Med* 1993; **23**: 437-451
12 **Lee AM**, Lee S. Disordered eating and its psychosocial correlates among Chinese adolescent females in Hong Kong. *Int J Eat Disord* 1996; **20**: 177-183
13 **Chisuwa N**, O'Dea JA. Body image and eating disorders amongst Japanese adolescents. A review of the literature. *Appetite* 2010; **54**: 5-15
14 **Nasser M**. Screening for abnormal eating attitudes in a population of Egyptian secondary school girls. *Soc Psychiatry Psychiatr Epidemiol* 1994; **29**: 25-30
15 **Buhrich N**. Frequency of presentation of anorexia nervosa in Malaysia. *Aust N Z J Psychiatry* 1981; **15**: 153-155
16 **Abrams KK**, Allen LR, Gray JJ. Disordered eating attitudes and behaviors, psychological adjustment, and ethnic identity: a comparison of black and white female college students. *Int J Eat Disord* 1993; **14**: 49-57
17 **Kumanyika S**, Wilson JF, Guilford-Davenport M. Weight-related attitudes and behaviors of black women. *J Am Diet Assoc* 1993; **93**: 416-422
18 **Arriaza CA**, Mann T. Ethnic differences in eating disorder symptoms among college students: the confounding role of body mass index. *J Am Coll Health* 2001; **49**: 309-315
19 **Rodin GM**, Daneman D. Eating disorders and IDDM. A problematic association. *Diabetes Care* 1992; **15**: 1402-1412
20 **Colton PA**, Rodin GM, Olmsted MP, Daneman D. Eating disturbances in young women with type 1 diabetes mellitus: mechanisms and consequences. *Psychiatr Ann* 1999; **29**: 213-218
21 **Polivy J**, Herman CP. Dieting and binging. A causal analysis. *Am Psychol* 1985; **40**: 193-201
22 **Collazo-Clavell M**. Diabulimia: How are diabetes and eating disorder related? 2007. Available from: URL: http://www.Mayoclinic.com/health/diabulimia/ANO1737
23 **Mathur R**, Conrad M. Diabulimia-Eating disorder. 2008. Available from: URL: http://www.medicinenet.com/script/main/art.asp
24 **Bryden KS**, Neil A, Mayou RA, Peveler RC, Fairburn CG, Dunger DB. Eating habits, body weight, and insulin misuse. A longitudinal study of teenagers and young adults with type 1 diabetes. *Diabetes Care* 1999; **22**: 1956-1960
25 A battle to overcome "diabulimia". *Am Fam Physician* 2009; **79**: 263; discussion 263
26 **Hasken J**, Kresl L, Nydegger T, Temme M. Diabulimia and the role of school health personnel. *J Sch Health* 2010; **80**: 465-469; quiz 514-516
27 **Ruth-Sahd LA**, Schneider M, Haagen B. Diabulimia: what it is and how to recognize it in critical care. *Dimens Crit Care Nurs* 2009; **28**: 147-153; quiz 154-155
28 **Neumark-Sztainer D**, Patterson J, Mellin A, Ackard DM, Utter J, Story M, Sockalosky J. Weight control practices and disordered eating behaviors among adolescent females and males with type 1 diabetes: associations with sociodemographics, weight concerns, familial factors, and metabolic outcomes. *Diabetes Care* 2002; **25**: 1289-1296
29 **Rydall AC**, Rodin GM, Olmsted MP, Devenyi RG, Daneman D. Disordered eating behavior and microvascular complications in young women with insulin-dependent diabetes mellitus. *N Engl J Med* 1997; **336**: 1849-1854
30 **Affenito SG**, Adams CH. Are eating disorders more prevalent in females with type 1 diabetes mellitus when the impact of insulin omission is considered? *Nutr Rev* 2001; **59**: 179-182
31 **Figueroa Sobrero A**, Evangelista P, Mazza C, Basso P, López SM, Scaiola E, Honf M, Ferraro M, Eandi ML, Walz F. [Three-year follow up of metabolic control in adolescents with type 1 diabetes with and without eating disorders]. *Arch Argent Pediatr* 2010; **108**: 130-135
32 **Crow SJ**, Keel PK, Kendall D. Eating disorders and insulin-dependent diabetes mellitus. *Psychosomatics* 1998; **39**: 233-243
33 **Rodin GM**, Craven J, Littlefield C, Goldbloom D, Daneman D. Insulin misuse through omission or reduction of dose. *Psychosomatics* 1989; **30**: 465-466
34 **Peveler RC**, Bryden KS, Neil HA, Fairburn CG, Mayou RA, Dunger DB, Turner HM. The relationship of disordered eating habits and attitudes to clinical outcomes in young adult females with type 1 diabetes. *Diabetes Care* 2005; **28**: 84-88
35 **Vila G**, Nollet-Clemencon C, Vera L, Crosnier H, Robert JJ, Mouren-Simeoni MC. [Eating disorders in an adolescent population with insulin-dependent diabetes]. *Can J Psychiatry* 1993; **38**: 606-610
36 **Friedman S**, Vila G, Timsit J, Boitard C, Mouren-Simeoni MC. [Eating disorders and metabolic balance in a population++ of young adults with insulin-dependent diabetes]. *Ann Med Psychol* (Paris) 1995; **153**: 282-285
37 **Jones JM**, Lawson ML, Daneman D, Olmsted MP, Rodin G. Eating disorders in adolescent females with and without type 1 diabetes: cross sectional study. *BMJ* 2000; **320**: 1563-1566
38 **Affenito SG**, Backstrand JR, Welch GW, Lammi-Keefe CJ, Rodriguez NR, Adams CH. Subclinical and clinical eating disorders in IDDM negatively affect metabolic control. *Diabetes Care* 1997; **20**: 182-184
39 **Affenito SG**, Rodriguez NR, Backstrand JR, Welch GW, Adams CH. Insulin misuse by women with type 1 diabetes mellitus complicated by eating disorders does not favorably change body weight, body composition, or body fat distribution. *J Am Diet Assoc* 1998; **98**: 686-688
40 **Polonsky WH**, Anderson BJ, Lohrer PA, Aponte JE, Jacobson AM, Cole CF. Insulin omission in women with IDDM. *Diabetes Care* 1994; **17**: 1178-1185
41 **Steel JM**, Young RJ, Lloyd GG, Clarke BF. Clinically apparent eating disorders in young diabetic women: associations with painful neuropathy and other complications. *Br Med J* (Clin Res Ed) 1987; **294**: 859-862
42 **Colas C**. Eating disorders and retinal lesions in type 1 (insulin-dependent) diabetic women. *Diabetologia* 1991; **34**: 288
43 **Ward A**, Troop N, Cachia M, Watkins P, Treasure J. Doubly disabled: diabetes in combination with an eating disorder. *Postgrad Med J* 1995; **71**: 546-550
44 The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med* 1993; **329**: 977-986
45 **Walker JD**, Young RJ, Little J, Steel JM. Mortality in concurrent type 1 diabetes and anorexia nervosa. *Diabetes Care* 2002; **25**: 1664-1665
46 **Goebel-Fabbri AE**, Fikkan J, Franko DL, Pearson K, Anderson BJ, Weinger K. Insulin restriction and associated morbid-

ity and mortality in women with type 1 diabetes. *Diabetes Care* 2008; **31**: 415-419
47 **Krakoff DB**. Eating disorders as a special problem for persons with insulin-dependent diabetes mellitus. *Nurs Clin North Am* 1991; **26**: 707-713
48 **DAFNE Study Group**. Training in flexible, intensive insulin management to enable dietary freedom in people with type 1 diabetes: dose adjustment for normal eating (DAFNE) randomised controlled trial. *BMJ* 2002; **325**: 746
49 **Trento M**, Borgo E, Kucich C, Passera P, Trinetta A, Charrier L, Cavallo F, Porta M. Quality of life, coping ability, and metabolic control in patients with type 1 diabetes managed by group care and a carbohydrate counting program. *Diabetes Care* 2009; **32**: e134
50 **Goebel-Fabbri AE**. Diabetes and eating disorders. *J Diabetes Sci Technol* 2008; **2**: 530-532
51 **Russell-Jones D**, Khan R. Insulin-associated weight gain in diabetes--causes, effects and coping strategies. *Diabetes Obes Metab* 2007; **9**: 799-812
52 **Anzai N**, Lindsey-Dudley K, Bidwell RJ. Inpatient and partial hospital treatment for adolescent eating disorders. *Child Adolesc Psychiatr Clin N Am* 2002; **11**: 279-309
53 **American Diabetes Association**. Standards of medical care for patients with diabetes mellitus. *Diabetes Care* 2001; **24** (Suppl 1): S33-S43
54 **Goebel-Fabbri AE**. Disturbed eating behaviors and eating disorders in type 1 diabetes: clinical significance and treatment recommendations. *Curr Diab Rep* 2009; **9**: 133-139
55 **Rosen DS**. Eating disorders in children and young adolescents: etiology, classification, clinical features, and treatment. *Adolesc Med* 2003; **14**: 49-59
56 **Walsh JM**, Wheat ME, Freund K. Detection, evaluation, and treatment of eating disorders the role of the primary care physician. *J Gen Intern Med* 2000; **15**: 577-590
57 **Stewart TM**, Williamson DA, White MA. Rigid vs. flexible dieting: association with eating disorder symptoms in nonobese women. *Appetite* 2002; **38**: 39-44
58 **Daneman D**, Olmsted M, Rydall A, Maharaj S, Rodin G. Eating disorders in young women with type 1 diabetes. Prevalence, problems and prevention. *Horm Res* 1998; **50** Suppl 1: 79-86
59 **Daneman D**, Rodin G. Eating disorders in young women with type 1 diabetes: a cause for concern? *Acta Paediatr* 1999; **88**: 117-119
60 **Kelly SD**, Howe CJ, Hendler JP, Lipman TH. Disordered eating behaviors in youth with type 1 diabetes. *Diabetes Educ* 2005; **31**: 572-583
61 **Markowitz JT**, Butler DA, Volkening LK, Antisdel JE, Anderson BJ, Laffel LM. **Brief screening tool for disordered** eating in diabetes: internal consistency and external validity in a contemporary sample of pediatric patients with type 1 diabetes. *Diabetes Care* 2010; **33**: 495-500

S- Editor Wu X **L- Editor** Roemmele A **E- Editor** Zheng XM

World Journal of Diabetes

Online Submissions: http://www.wjgnet.com/1948-9358office
wjd@wjgnet.com
doi:10.4239/wjd.v2.i11.196

World J Diabetes 2011 November 15; 2(11): 196-203
ISSN 1948-9358 (online)

TOPIC HIGHLIGHT

Didier Vieau, Professor, *Series Editor*

Consequences of gestational and pregestational diabetes on placental function and birth weight

Anne Vambergue, Isabelle Fajardy

Anne Vambergue, EA 4489 "Perinatal Environment and Fetal Growth", Department of Diabetology, Huriez Hospital, 59800 CHRU Lille, France
Isabelle Fajardy, EA 4489 "Perinatal Environment and Fetal Growth", Biology and Pathology Center, 59800 CHRU Lille, France
Author contributions: Vambergue A and Fajardy I designed and wrote Part Ⅰ, Ⅱ, Ⅳ, Ⅴ and part Ⅲ respectively; all authors approved the final version of the topics (manuscript) to be published.
Correspondence to: **Anne Vambergue, Professor,** EA 4489 "Perinatal Environment and Fetal Growth", Department of Diabetology, Huriez Hospital, 59800 CHRU Lille, France. anne.vambergue@chru-lille.fr
Telephone: +33-320-446816 Fax: +33-320-444184
Received: March 18, 2011 Revised: October 19, 2011
Accepted: October 26, 2011
Published online: November 15, 2011

Abstract

Maternal diabetes constitutes an unfavorable environment for embryonic and fetoplacental development. Despite current treatments, pregnant women with pregestational diabetes are at increased risk for congenital malformations, materno-fetal complications, placental abnormalities and intrauterine malprogramming. The complications during pregnancy concern the mother (gravidic hypertension and/or preeclampsia, cesarean section) and the fetus (macrosomia or intrauterine growth restriction, shoulder dystocia, hypoglycemia and respiratory distress). The fetoplacental impairment and intrauterine programming of diseases in the offspring's later life induced by gestational diabetes are similar to those induced by type 1 and type 2 diabetes mellitus. Despite the existence of several developmental and morphological differences in the placenta from rodents and women, there are similarities in the alterations induced by maternal diabetes in the placenta from diabetic patients and diabetic experimental models. From both human and rodent diabetic experimental models, it has been suggested that the placenta is a compromised target that largely suffers the impact of maternal diabetes. Depending on the maternal metabolic and proinflammatory derangements, macrosomia is explained by an excessive availability of nutrients and an increase in fetal insulin release, a phenotype related to the programming of glucose intolerance. The degree of fetal damage and placental dysfunction and the availability and utilisation of fetal substrates can lead to the induction of macrosomia or intrauterine growth restriction. In maternal diabetes, both the maternal environment and the genetic background are important in the complex and multifactorial processes that induce damage to the embryo, the placenta, the fetus and the offspring. Nevertheless, further research is needed to better understand the mechanisms that govern the early embryo development, the induction of congenital anomalies and fetal overgrowth in maternal diabetes.

Key words: Maternal diabetes; Placental function; Birth weight; Macrosomia; Intrauterine growth retardation

Peer reviewers: David J Hill, Professor, Lawson Health Research Institute, 268 Grosvenor Street, London, Ontario N6A4V2, Canada; KVS Hari Kumar, Dr., Department of Endocrinology, Command Hospital, Central Command, Lucknow Cantt, Lucknow 226002, India

Vambergue A, Fajardy I. Consequences of gestational and pregestational diabetes on placental function and birth weight. *World J Diabetes* 2011; 2(11): 196-203 Available from: URL: http://www.wjgnet.com/1948-9358/full/v2/i11/196.htm DOI: http://dx.doi.org/10.4239/wjd.v2.i11.196

INTRODUCTION

Diabetes in pregnant women is associated with an in-

creased risk of maternal and neonatal morbidity and remains a significant medical challenge.

Diabetes during pregnancy may be divided into clinical diabetes (women with previously diagnosed with type 1 or type 2 diabetes) and gestational diabetes. The American Diabetes Association defines gestational diabetes as "any degree of glucose intolerance with onset or first recognition during pregnancy", but provides diagnostic thresholds for fasting and post-glucose loading values. The International Association of Diabetes in Pregnancy Study Groups recently published a consensus derived from the Hyperglycemia Adverse Pregnancy Outcome study data, suggested that all pregnant women without known diabetes should have a 75 g oral glucose tolerance test at 24-28 wk of gestation[1]. Gestational diabetes would be diagnosed if one or more values met or exceeded the following levels of glucose: fasting 5.1 mmol/L, 1 h post glucose 10.0 mmol/L and 2 h post glucose 8.5 mmol/L.

While diabetes in pregnancy is associated with increased obstetric risk compared with normal pregnancy, the overall contribution of diabetes to most obstetric and neonatal complications on a population basis is low, with the largest impact being on shoulder dystocia. Except malformations, which are likely to have resulted from preconceptional or periconceptional hyperglycemia, improvements in obstetric practice have led to major reductions in adverse outcomes. Prepregnancy care for women with diabetes was introduced a long time ago and is associated with improved pregnancy outcomes. However, overall pregnancy outcomes remain very poor for women with diabetes with only a third receiving prepregnancy care. The importance of other metabolic factors, such as obesity and hypertriglyceridemia, in pregnancy are also now increasingly being recognized.

As well as its effects on perinatal outcomes, the intrauterine environment is a key determinant of child and adult health, particularly of conditions associated with metabolic disturbances[2-4]. Aberrant fetal growth has been linked to the development of metabolic diseases in later life, with many authors describing J or U-shaped curves linking both low and high birth weights with these conditions[5,6].

The diabetic intrauterine environment affects the offspring of women with all types of diabetes mellitus (DM). Therefore, type 2 diabetes is very often associated with obesity. It is possible that changes in placental structure and function may exist in obesity independently of diabetes. But, at this time, we do not have sufficient data in the literature to distinguish between what is related to obesity or to diabetes. The offspring of women with diabetes during pregnancy is at higher risk of developing hypertension and other cardiovascular disease. The intrauterine environment represents a vicious cycle with the offspring being at risk of developing gestational diabetes or diabetes at a young age. More recently, it has been evocated that environmental signals can alter the epigenetic state of specific genes and modulate their activity. It is possible that modulation of epigenetic states provides a plausible mechanism by which maternal diabetes can mediate known long-term effects on risk for type 2 diabetes for the offspring, but we need more information to understand the concept of programming in maternal diabetes.

PLACENTA IN DIABETES

The placenta is located at the interface between the maternal and fetal circulation with fundamental functions for pregnancy. It has been postulated that the diabetic environment may have profound effects on placental development and function. It is very important to be precise that these specific effects will depend on the time period in gestation[7]. Maternal and fetal hyperglycemias are likely to have an impact on the production of various placental proteins. These maternal and fetal hyperglycemiae also affect placental metabolism, growth and development.

Despite the improvement in maternal glycemic control, structural and functional changes of the diabetic placenta at term may occur independently of the type of diabetes. In the case of diabetes, the surface area is particularly increased in the periphery of the villous tree. The diffusion distance between the maternal and fetal systemic circulations is increased due to a thickening of the trophoblastic basement membrane with higher amounts of collagen, predominantly type Ⅳ[8].

In type 1 diabetes and in gestational DM (GDM), the villous stroma is slightly edematous with an over-representation of Hofbauer cells which are the placental resident macrophages[9]. The increased number of these cells contributes to a higher release of placental cytokines such as leptin, tumor necrosis factor-α (TNF-α) and interleukins, and subsequently modifies placental metabolic and endocrine functions[10]. Enlargement of the capillary surface area with capillary proliferation and penetration of newly formed vessels has been also described in maternal diabetes[11]. So the result is a hyper-vascularization and an increased surface of exchange that could contribute to oxygen diffusion across the placenta to compensate for the impaired maternal-fetal transfer of diffusion-limited substances.

Placental weight tends to be heavier in diabetes, similar to fetal weight, but the weight gain is more pronounced in the placenta than in the fetus, as is reflected in a higher placental-to-fetal weight ratio than in normal gestation. Placentomegaly is correlated with fetal macrosomia confirming the close correlation of placental weight with that of the offspring[12]. Actually, it is not possible to determine if placental overweight is the cause or the consequence of fetal overweight.

Modification in placental transport

Despite altered expression of placental glucose transporters, an unchanged transplacental glucose transport in GDM has been demonstrated. Taricco *et al*[13] showed that there was an unchanged concentration difference for glucose in umbilical arteries and veins in GDM. The higher flux results from the steeper maternal-to-fetal

concentration gradient as the major reason for increased glucose across the placenta in diabetes.

Amino acid transport may be also altered in diabetes[14]. Changes in placental amino acid transporters are not associated with maternal diabetes, but rather with elevated fetal weight[15]. We do not know if increased nutrient transport will stimulate fetal growth or serve to cover the increased fetal nutrient in case of overgrowth of the fetus.

Modification in materno-placental oxygen supply

The placental structure is altered in pregestational and gestational diabetes. The surface and exchange areas are enlarged as a result of hyperproliferation and hypervascularization. The underlying mechanisms are unclear but we can not exclude the role of the maternal hyperglycemia associated with other maternal factors[16].

In diabetes, it has been shown that the maternal-placental oxygen supply is reduced[17]. In addition to impaired oxygen supply, fetal oxygen demand is increased. This phenomenon could be explained by aerobic metabolism which is stimulated by fetal hyperinsulinemia. The resulting low fetal oxygen levels upregulate the transcription synthesis of proangiogenic factors such as leptin, vascular endothelial growth factor (VEGF) or fibroblast growth factor 2 (FGF2)[18,19]. In excess, these factors promote placental endothelial cell proliferation. In GDM and in type 1 DM (T1DM), alterations in fetal levels of proangiogenic [VEGF, FGF2, leptin, OGF1, insulin-like growth factor (IGF)2, hypoxia] or anti-angiogenic (TNF-α) factors have been reported. Both types of diabetes are characterized by enhanced vascularisation.

The diabetic milieu will have an influence on placental development and function especially during the first trimester. In this period, the placental structures are formed and the placenta is more sensitive to modifications. The hyperglycemia can induce a reduction of trophoblast proliferation which delays placental growth and development, especially in the first gestational weeks[20]. This mechanism could explain the higher incidence of spontaneous abortion, pre-eclampsia and intrauterine growth restriction associated with diabetes, and suggest impaired trophoblast invasion[21].

More recently, it has been shown that placental expression and activity of the matrix metalloproteinases (MMPs) as MMP14 and MMP15 are elevated in diabetes, especially in type 1 diabetes induced by maternal hyperinsulinemia and TNF-α[21]. MMP14 and MMP15 are proteases which are involved in tissue remodeling processes associated with invasion, angiogenesis and proliferation. The active form of placental MMP14 is elevated in diabetes. It is possible that hypoxic situations in the villous placental structure may be implicated as a cause of increased MMP14 activity.

SUITABLE ANIMAL MODELS ARE NEEDED TO STUDY DIABETES DURING PREGNANCY

With regard to diabetes in pregnancy, experimental findings from animal models and particularly rodents were developed with the purpose of enhancing understanding of pathophysiological mechanisms and further finding treatment strategies to maintain the closest to normal metabolic intrauterine milieu, improving perinatal development by preventing fetal growth restriction or macrosomia. Resulting diabetes in various animal species, including sheep, pig and rabbit have been described in many reviews[22-25]. However, the most often used experimental models are rodents (Wistar rat) because of their convenient maintenance, short length of pregnancy and multiparity, thus enabling studies on multiple fetuses and generations. The aim of type 1 and type 2 animal models is to reproduce metabolic maternal and fetal effects similar to pregestational and GDM. But which is the most suitable model? It has been shown that children born from either pre-existing T1DM, type 2 DM (T2DM) and GDM are equally exposed to impaired glucose intolerance[26]. How to reproduce the more accurately metabolic maternal end neonatal effects of diabetes? What is the most efficient tool? The efficiency of an experimental model is based on the observation of a stable hyperglycemia throughout gestation.

We will first distinguish models of inducing maternal diabetes using chemical agents able to damage the maternal β cell with short term consequences in fetuses. Secondly, long term induction of metabolic diseases by nutritional changes occurring before and/or during pregnancy named fetal programming models. We will then expose genetically predetermined diabetes animal models.

Chemical models of diabetes during pregnancy

Chemical methods used to induce damage to the pancreatic β-cells are obtained through the administration of drugs such as streptozotocin (STZ).

STZ: STZ is a potent alkylating agent able to methylate DNA and is often used to induce diabetes in experimental animals due to its toxic effects on pancreatic β-cells[27]. The nitrosurea moiety of STZ is responsible for its cellular toxicity, which is probably mediated through a decrease in nicotinamide (NAD) levels and the production of intracellular free radicals. Beta cells are particularly sensitive to STZ due to their low level of NAD. Many different approaches have been used regarding the mode of injection (intravenous or intraperitoneal), the doses (30 to 50 mg/kg) and the stage (pre gestational and gestational) leading to severe or mild diabetes and subsequent opposite fetal phenotypes (intra-uterine growth restriction or macrosomic[28,29]). In fact, mildly diabetic dams are hyperglycemic (glycemia between 120 and 300 mg/dL), hypoinsulinemic and give birth to macrosomic fetuses. In contrast, severely diabetic mothers are insulin deficient, hyperglycemic with low body weight and give birth to microsomic and malformed fetuses. The mechanisms are a brutal destruction of the mother and fetus's pancreas and intrauterine growth restriction is due to lack of insulin[30]. The altered maternal-fetal metabolic fuel relationship resulting from diabetes in pregnancy modulates fetal growth.

The increase in fetal glucose and insulin availability with maternal diabetes is strongly associated with the development of fetal macrosomia but severe DM or diabetes of long duration restricts fetal growth. Rat fetal body weight correlates positively with maternal glucose in diabetic rats with a glucose level less than 220 mg/dL but it correlates negatively in rats with a level above 220 mg/dL. Merzouk and Soulimane-Mokhtari[31] verified that mildly hyperglycemic dams have fetuses that are large for gestational age, classified as macrosomic, but this data has been hardly reproduced in other studies[32]. In 2010, other authors observed an increase of placental weight in their mildly diabetic rats showing a compensatory mechanism to assure the maternal fetal exchanges contribution to fetal development[33]. The degree of fetal damage and placental dysfunction and the availability and utilization of fetal substrates can lead to the induction of macrosomia or microsomia. We observed a U-shaped relationship between offspring weight and metabolic changes, like in clinical studies.

STZ + NAD chemical model: NAD is an anti oxidant molecule that protects the β cell destruction induced by STZ. In 1998, Masiello and co-workers developed NAD-STZ diabetes model induced by STZ and partially protected with a suitable dose of NAD[34]. In this NAD-STZ model, the diabetic syndrome shares a number of features with human T2DM such as a stable moderate hyperglycemia, glucose intolerance, an altered insulin secretion and a reduction in pancreatic β cell mass. So a mild diabetic model might be induced by STZ under NAD protection.

Using STZ in experimental models: Chemical models are characterized by a toxic action against β cells leading to a destruction of producing insulin cell without insulin resistance. These models mimic a type 1 diabetic state, despite the lack of immunological and genetic disorders. Thus, we obtain a type 1 maternal diabetes quite different from gestational diabetes which is characterized by a lack of adaptation of β cells to metabolic changes occurring during pregnancy and by an enhanced resistance to insulin.

Nevertheless, some authors have tried to mimic gestational diabetes by varying the doses and the window of injection of STZ (in the neonatal period[35,36], before mating[37] or during pregnancy[38]).

Drawbacks of chemical models: The efficiency of a model is based on the observation of a stable hyperglycemia throughout gestation and depends on multiple parameters such as the dose and mode of injection (venous or peritoneal route)[38]. Moreover, the efficiency varies according to the sex, with a lower metabolic sensibility in females[39]. The authors reported a sexual dimorphism in insulin sensibility with females being less sensitive to insulin than males, leading to a higher susceptibility to the rapid development of a more severe form of diabetes. Females have more islets compared to males: higher insulin rates and lower glucose rates and rat male pancreatic β cells are protected by testosterone against STZ induced apoptosis[40]. In light of these data, it is clear that we have to take into account these variations in experimental model settings.

Dietary interventions

Exposure to an adverse environment *in utero* programs the physiology and metabolism of the offspring permanently with long term consequences for health[41]. This is the basis of the thrifty phenotype hypothesis driven from epidemiological studies showing a strong statistical link between birth weight, further metabolic syndrome and maternal nutrition in a context of type 2 diabetes[42]. Nutrition is a key environmental factor and it has been shown that inappropriate nutrition *in utero* has consequences into adulthood[43]. We speak about nutritional programming as the effect that occurred long after the stressor has been removed. Beta cell development is irreversibly damaged by inadequate nutrition during critical periods of fetal development[44]. This is a fetus organogenesis adaptation to the fetal-placenta unit environment.

Others studies suggest that the post natal period, when catch up growth occurs, may be more important than the *in utero* period[45]. The two arguments are now taken into account to suggest that a combination of intrauterine deprivation followed by accelerated post natal growth induce the highest risk of further metabolic disease. In other words, increased risk of disease arises if there is an imbalance between pre natal and post natal nutritional uptake. This is the basis of two steps nutritional models.

We will consider four main nutritional models: high fat, low protein, high carbohydrate and two steps nutritional programming model.

High fat diet model: In humans, consuming a high fat diet model (HFD) causes an increase in body fat deposition and a decrease in insulin sensibility which leads to insulinoresistance and T2DM.

HFD diet is more representative of the eating habits of the current society in both the developing and westernized world. Diets rich in saturated fats before and during pregnancy may result in pathological manifestations in rodents similar to the human condition of GDM.

Ozaki *et al*[46] have shown that a diet that includes 20% fat during pregnancy changes blood pressure in the offspring. This is explained by alterations in the fatty acid profiles of the membrane of the aorta. Fatty acid oxidation inhibits both glucose oxidation and its ability to enter cells. HFD increases insulin resistance in rats by secretion of cytokines and TNF in several tissues[47].

Rats fed with HFD develop obesity, hyperinsulinemia and insulin resistance but not frank hyperglycemia and diabetes. HFD impairs the glucose signaling system of the β cell and the capacity of insulin secretion and leads to a reduction of β cell mass and an increased apoptosis[48].

Otherwise, by increasing the fat component in the diet, the levels of the other macronutrients would be also affected, leading to nutrient deficiencies.

Low protein model: Low protein diet exposure during the first 3 or 6 wk of life in rats have consequences on growth and insulin secretory response[49]. During pregnancy, these animals are unable to match or adapt to insulin request and become glucose intolerant. Authors concluded that temporary protein energy malnutrition in young rats reduces the ability to increase insulin production to meet the needs of pregnancy.

Glucose regulation is perturbed and glucose and other nutrients are transferred to fetuses in increased amounts. This stimulates pancreatic β cell growth and insulin secretion and thus the occurrence of macrosomia in the offspring. The length of diet exposure has an impact on fetus development and physiology. A diet restricted to the first week of gestation leads to hypoglycemic and low weight fetuses with a high risk of further T2DM[50]. Diet during complete gestation leads to hyperglycemic fetuses and permanent β cell alterations such as reducing β cell volume and content and consequently the capacity of insulin secretion[51].

Carbohydrate model: Infusions of glucose during pregnancy in rats lead to a transient hyperglycemia[52]. However, significant effects on fetuses need repeated injections of glucose and the window of injection is important with a positive effect restricted to early pregnancy. This finding matches clinical observations of macrosomia despite a good glycemic control in second trimester. This suggests that metabolic control in early pregnancy is an important determinant for fetal-placental growth throughout gestation.

Two steps programming nutritional models: We consider a first step of restrictive diet (food restriction, FR30) occurring at the first generation during the 1st period of life and gestation, and a second step of high caloric diet submitted to the offspring after birth and weaning. Fetuses of this second generation will develop a metabolic syndrome[53].

Pregnant genetically determined diabetes

Pregnant animals with genetically determined type 1 diabetes: The Non Obese Diabetic (NOD) mice and Bio Breeding (BB) rats develop spontaneous pre-gestational diabetes and thus represent good candidates for type 1 maternal diabetes models. In common with human diabetes, β cells are submitted to an immune attack and animals have to be treated with insulin during gestation. Stopping insulin treatment induces a loss of maternal weight, ketosis, high rate of fetus resorption, lower fetal weight and higher placental weight[54]. Therefore, BB rats are a good model for the study of perinatal morbidity, microsomia and malformations. NOD mice develop a mild form of diabetes with macrosomic fetuses and adiposity. It has been shown that maternal hyperglycemia is not the only causative factor of macrosomia, partially explained by a dysregulation of placental glucose transporters and hexokinase protein production unable to protect the fetus from hyperglycemia and hyperinsulinemia[55].

Pregnant animals with genetically determined type 2-like diabetes: There are models of obesity and diabetes affecting a common pathway, a defect in the leptin receptor (*db*) and a defect in the leptin gene (*ob*). The deficiency of leptin has consequences in multiple areas of metabolism, ingestive behavior and reproduction (insulin resistance, hyperphagia and infertility). Most of these experimental models at the homozygous state are infertile. Therefore, mating heterozygotes animals is necessary to inbreeding. Three main models exist: C57 BIKS Lepr $^{db+}$and C57Bl/6j mice and Goto-Kakizaki rats. Heterozygous Lepr $^{db+}$ mice are normoglycemic before pregnancy and present significant glucose intolerance restricted to the gestation period and associated with fetal macrosomia[56]. After delivery, glycemia reverts to a normal state, making Lepr db mice a good model for GDM investigation. C57BL/6j mice develop diabetes only after a high-fat and sucrose-rich diet. This model induced diabetes and obesity with hyperinsulinemia and hyperlipidemia, and females return to normal weight and glucose tolerance after gestation[57]. *Goto Kakizaki* (*GK*) is a rodent model of non obese type 2 diabetes that was produced by selective breeding of individuals with mild glucose intolerance from a non diabetic Wistar rat colony. A stable and heritable DM is obtained by selection of a diabetic line isolated by repeated breeding of normal animals[58]. These rats are not obese and not hyperinsulinemic. Offspring of GK females is exposed *in utero* to mild diabetes throughout gestation. Fetuses present a reduced β-cell mass associated with a lack of pancreatic reactivity to glucose. Thus, maternal mild hyperglycemia might contribute to endocrine pancreas defects in the first offspring generation.

ROLE OF THE INSULIN/INSULIN-LIKE GROWTH FACTORS SYSTEM

The insulin/IGF system is implicated in the regulation of fetal and placental growth and development. The feto-placental expression of insulin, IGF1, IGF2 and their receptors is regulated in a tissue-specific manner and can be affected by nutritional and endocrine conditions[59]. Hiden *et al*[60] have demonstrated that there is a spatio-temporal change in placental insulin receptor (IR) expression, suggesting a shift in the regulation of placental insulin effects from mother to fetus. In the first trimester, IR is predominantly expressed on the syncytiotrophoblast facing the maternal circulation, whereas at term, the placental endothelial cells facing the fetal circulation are the main expression site. The placental IGF1 receptor (IGF1R) is mainly expressed on the basal membrane of the syncytiotrophoblast. Hence, it is predominantly accessible for fetal IGF1 and IGF2[61]. *IGF2* overexpression

enhanced fetal growth, whereas targeted disruption of the fetal *IGF1*, *IGF2* or *IGF1R* genes in mice resulted in retardation of fetal growth.

The IGF-binding proteins (IGFBPs) are important players in the IGF system. They are key modulators of the ligand-receptor interaction. In humans, the most prevalent IGFBPs in fetal plasma and tissue are the IGFBPs 1-4. The serum of pregnant women contains the placenta-derived IGFBP3 protease. It cleaves IGFBP3 into smaller fragments with lower affinities for IGFs. Decidual cells of the basal plate region express mRNA of all six IGFBPs in the second and third trimester, with IGFBP1 being the most abundant. In the placenta, IGFBP3 is expressed in the extravillous cytiotrophoblasts. Fetal cord blood data suggest that these binding proteins may be dysregulated by diabetes during pregnancy[62]. *IGFBP3* mRNA is increased in maternal T1DM[63]. In the cord blood, increased IGFBP3 levels correlate with IGF1 levels and the incidence of macrosomia. This is consistent with the observation that IGF1 and IGFBP3 levels directly correlate with birth weight in diseased states[64].

The endocrine interaction between mother, fetus and placenta is exemplified by the effect of maternal and fetal insulin on the placenta. Maternal insulin affects placental development *via* receptors expressed on the microvillous membrane of the syncytiotrophoblast[21]. Fetal insulin affects gene expression in endothelial cells from placental arteries and veins, which will affect placental development. The spatio-temporal change of IR expression in the placenta allows a shift in the control of insulin regulation from the mother to the fetus. In the first trimester, maternal insulin influences the placenta by interaction with trophoblast IRs. This may in turn affect the mother by secretion of other factors as cytokines and hormones. Later, the fetus takes over control of insulin-dependent placental processes by fetal insulin interacting with placental endothelial cells.

In addition, in the first trimester, IGF1 and IGF2 produced by trophoblasts stimulate various processes that are involved in trophoblast invasion into the maternal uterus such as invasiveness, migration, MMP2 production, proliferation and MT1-MMP expression. Lower maternal IGF1 levels in T1DM may thus contribute to impaired trophoblast invasion. Hence, in GDM, transplacental amino acid transport and fetal growth may be promoted by the diabetes-associated increase in maternal concentrations of growth factors. TNF-α inhibits trophoblast invasion, whereas VEGF, leptin, insulin, IGF1 and IGF2 promote trophoblastic invasion. Changes can also be seen in the fetal circulation. However, the consequences of these changes for the fetus remain unclear.

ROLE OF LEPTIN

Maternal and fetal hyperleptinemia are well-established in diabetes and obesity. The extensive cross-talk between insulin and leptin signaling cascades may represent a major factor to the diabetes-induced placental changes. In humans, leptin levels correlate with adiposity. This hormone has different functions such as stimulation of angiogenesis, regulation of hematopoiesis and inflammatory response[65]. During gestation, maternal leptin concentration rises by 30% and the placenta becomes the primary leptin source. The leptin receptor is expressed in the syncytiotrophoblast. Hauguel-de Mouzon *et al*[65] have shown that leptin induces hCG production, enhances mitogenesis, stimulates amino acid uptake and increases the synthesis of extracellular matrix proteins and metalloproteinases. So leptin plays a role in the regulation of placental growth. However, hyperleptinemia contributes to other placental modifications in the case of diabetes as basement membrane thickening owing to its ability to alter collagen synthesis[66].

Hiden *et al*[67] have proposed a hypothetical model for diabetes-induced alterations in human placenta. Elevated maternal TNF-α and reduced IGF1 levels in T1DM may inhibit placental invasion, paralleling a higher incidence of early pregnancy loss in diabetes. Maternal hyperglycemia induces thickening of the placental basement membrane, hence reducing oxygen transport. Increased levels of placental leptin may even further contribute to the excessive extracellular matrix synthesis. Different factors elevated in the placenta (IGF2, leptin), maternal (insulin, VEGF) or fetal (insulin, IGF1, IGF2, leptin) circulations in diabetes promote proliferation and placental growth. Placental hypervascularization may be supported by elevated levels of placental IGF2 and leptin, increased fetal IGF1, IGF2, leptin, FGF2, and reduced TNF-α, as well as fetal hypoxia. These modifications in the feto-placental compartment are characteristic of GDM, overt diabetes or both[67].

CONCLUSION

Placental structure and function can be changed as a result of maternal diabetes. The nature and extent of these changes depend on the type of diabetes and on the gestational period. For a complex disease syndrome, no animal model can be expected to serve all needs of research. Although each animal model has limitations and strengths, used together in a complementary fashion, they are essential for research on the metabolic syndrome and for rapid progress in understanding the etiology and pathogenesis towards a cure. Animal models have shown convincingly that diabetes may be transmitted by intrauterine exposure to maternal hyperglycemia. Intrauterine exposure to mild hyperglycemia is associated with normal weight or macrosomic newborns and IGT at adult age, related to a deficient insulin secretion. In contrast, a newborn offspring of severely hyperglycemic mothers is microsomic and displays, at adult age, a decreased insulin action. In addition, long-term and persistent effects of gestational diabetes on glucose homeostasis in the offspring may be transmitted through generations. These data support the concept of programming of physiological metabolism in offspring by manipulating maternal nutrition. It is known

that hyperglycemia is not the only causal factor. Maternal and fetal concentrations of several growth factors, hormones and cytokines are altered in diabetes and may affect the placenta and the fetal development. It is thus necessary to identify the specific biological effects and the mechanisms underlying them.

REFERENCES

1 **Metzger BE**, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, Dyer AR, Leiva A, Hod M, Kitzmiler JL, Lowe LP, McIntyre HD, Oats JJ, Omori Y, Schmidt MI. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. *Diabetes Care* 2010; **33**: 676-682

2 **Boney CM**, Verma A, Tucker R, Vohr BR. **Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus.** *Pediatrics* 2005; **115**: e290-e296

3 **Catalano PM**. Obesity and pregnancy--the propagation of a viscous cycle? *J Clin Endocrinol Metab* 2003; **88**: 3505-3506

4 **Simmons R**. Developmental origins of adult metabolic disease. *Endocrinol Metab Clin North Am* 2006; **35**: 193-204, viii

5 **Grattan DR**. Fetal programming from maternal obesity: eating too much for two? *Endocrinology* 2008; **149**: 5345-5347

6 **McCance DR**, Pettitt DJ, Hanson RL, Jacobsson LT, Knowler WC, Bennett PH. Birth weight and non-insulin dependent diabetes: thrifty genotype, thrifty phenotype, or surviving small baby genotype? *BMJ* 1994; **308**: 942-945

7 **Desoye G**, Hauguel-de Mouzon S. The human placenta in gestational diabetes mellitus. The insulin and cytokine network. *Diabetes Care* 2007; **30** Suppl 2: S120-S126

8 **Leushner JR**, Tevaarwerk GJ, Clarson CL, Harding PG, Chance GW, Haust MD. Analysis of the collagens of diabetic placental villi. *Cell Mol Biol* 1986; **32**: 27-35

9 **Sutton LN**, Mason DY, Redman CW. Isolation and characterization of human fetal macrophages from placenta. *Clin Exp Immunol* 1989; **78**: 437-443

10 **Varastehpour A**, Radaelli T, Minium J, Ortega H, Herrera E, Catalano P, Hauguel-de Mouzon S. Activation of phospholipase A2 is associated with generation of placental lipid signals and fetal obesity. *J Clin Endocrinol Metab* 2006; **91**: 248-255

11 **Sherer DM**, Divon MY. Prenatal ultrasonographic assessment of the ductus arteriosus: a review. *Obstet Gynecol* 1996; **87**: 630-637

12 **Taricco E**, Radaelli T, Nobile de Santis MS, Cetin I. Foetal and placental weights in relation to maternal characteristics in gestational diabetes. *Placenta* 2003; **24**: 343-347

13 **Taricco E**, Radaelli T, Rossi G, Nobile de Santis MS, Bulfamante GP, Avagliano L, Cetin I. Effects of gestational diabetes on fetal oxygen and glucose levels in vivo. *BJOG* 2009; **116**: 1729-1735

14 **Jansson T**, Ekstrand Y, Björn C, Wennergren M, Powell TL. Alterations in the activity of placental amino acid transporters in pregnancies complicated by diabetes. *Diabetes* 2002; **51**: 2214-2219

15 **Kuruvilla AG**, D'Souza SW, Glazier JD, Mahendran D, Maresh MJ, Sibley CP. Altered activity of the system A amino acid transporter in microvillous membrane vesicles from placentas of macrosomic babies born to diabetic women. *J Clin Invest* 1994; **94**: 689-695

16 **Mandl M**, Haas J, Bischof P, Nöhammer G, Desoye G. Serum-dependent effects of IGF-I and insulin on proliferation and invasion of human first trimester trophoblast cell models. *Histochem Cell Biol* 2002; **117**: 391-399

17 **Madsen H**, Ditzel J. Blood-oxygen transport in first trimester of diabetic pregnancy. *Acta Obstet Gynecol Scand* 1984; **63**: 317-320

18 **Jośko J**, Mazurek M. Transcription factors having impact on vascular endothelial growth factor (VEGF) gene expression in angiogenesis. *Med Sci Monit* 2004; **10**: RA89-RA98

19 **Black SM**, DeVol JM, Wedgwood S. Regulation of fibroblast growth factor-2 expression in pulmonary arterial smooth muscle cells involves increased reactive oxygen species generation. *Am J Physiol Cell Physiol* 2008; **294**: C345-C354

20 **Weiss U**, Cervar M, Puerstner P, Schmut O, Haas J, Mauschitz R, Arikan G, Desoye G. Hyperglycaemia in vitro alters the proliferation and mitochondrial activity of the choriocarcinoma cell lines BeWo, JAR and JEG-3 as models for human first-trimester trophoblast. *Diabetologia* 2001; **44**: 209-219

21 **Hiden U**, Glitzner E, Ivanisevic M, Djelmis J, Wadsack C, Lang U, Desoye G. MT1-MMP expression in first-trimester placental tissue is upregulated in type 1 diabetes as a result of elevated insulin and tumor necrosis factor-alpha levels. *Diabetes* 2008; **57**: 150-157

22 **Baird JD**, Aerts L. Research priorities in diabetic pregnancy today: the role of animal models. *Biol Neonate* 1987; **51**: 119-127

23 **Susa JB**, Boylan JM, Sehgal P, Schwartz R. Impaired insulin secretion after intravenous glucose in neonatal rhesus monkeys that had been chronically hyperinsulinemic in utero. *Proc Soc Exp Biol Med* 1992; **199**: 327-331

24 **Jovanovic L**, Kitzmiller JL, Peterson CM. Randomized trial of human versus animal species insulin in diabetic pregnant women: improved glycemic control, not fewer antibodies to insulin, influences birthweight. *Am J Obstet Gynecol* 1992; **167**: 1325-1330

25 **McNeill JH**. Experimental Models of Diabetes. *N Engl J Med* 2000; **342**: 63-64

26 **Burguet A**. Long-term outcome in children of mothers with gestational diabetes. *Diabetes Metab* 2010; **36**: 682-694

27 **Sybulski S**, Maughan GB. Use of streptozotocin as diabetic agent in pregnant rats. *Endocrinology* 1971; **89**: 1537-1540

28 **Aerts L**, Van Assche FA. **Endocrine pancreas in the offspring** of rats with experimentally induced diabetes. *J Endocrinol* 1981; **88**: 81-88

29 **Aerts L**, Holemans K, Van Assche FA. Impaired insulin response and action in offspring of severely diabetes rats. In: Shafir E, editor. Frontiers in Diabetes Research. Lessons from Animal Diabetes III. London: Slmith Godon, 1990: 561-566

30 **Schaffer SW**, Mozaffari MS. The neonatal STZ model of diabetes. In: McNeill JH, editor. Experimental models of diabetes. Boca Raton, FL: CRC Press, 1999: 231-255

31 **Soulimane-Mokhtari NA**, Guermouche B, Yessoufou A, Saker M, Moutairou K, Hichami A, Merzouk H, Khan NA. Modulation of lipid metabolism by n-3 polyunsaturated fatty acids in gestational diabetic rats and their macrosomic offspring. *Clin Sci* (Lond) 2005; **109**: 287-295

32 **Kiss AC**, Lima PH, Sinzato YK, Takaku M, Takeno MA, Rudge MV, Damasceno DC. Animal models for clinical and gestational diabetes: maternal and fetal outcomes. *Diabetol Metab Syndr* 2009; **1**: 21

33 **Bobadilla RA**, van Bree R, Vercruysse L, Pijnenborg R, Verhaeghe J. Placental effects of systemic tumour necrosis factor-α in an animal model of gestational diabetes mellitus. *Placenta* 2010; **31**: 1057-1063

34 **Masiello P**, Broca C, Gross R, Roye M, Manteghetti M, Hillaire-Buys D, Novelli M, Ribes G. Experimental NIDDM: development of a new model in adult rats administered streptozotocin and nicotinamide. *Diabetes* 1998; **47**: 224-229

35 **Blondel O**, Portha B. Early appearance of in vivo insulin resistance in adult streptozotocin-injected rats. *Diabete Metab* 1989; **15**: 382-387

36 **Tsuji K**, Taminato T, Usami M, Ishida H, Kitano N, Fukumoto H, Koh G, Kurose T, Yamada Y, Yano H. Characteristic features of insulin secretion in the streptozotocin-induced NIDDM rat model. *Metabolism* 1988; **37**: 1040-1044

37 **Caluwaerts S**, Holemans K, van Bree R, Verhaeghe J, Van Assche FA. Is low-dose streptozotocin in rats an adequate model for gestational diabetes mellitus? *J Soc Gynecol Investig* 2003; **10**: 216-221

38 **López-Soldado I**, Herrera E. Different diabetogenic response to moderate doses of streptozotocin in pregnant rats, and its long-term consequences in the offspring. *Exp Diabesity Res* 2003; **4**: 107-118

39 **Vital P**, Larrieta E, Hiriart M. Sexual dimorphism in insulin sensitivity and susceptibility to develop diabetes in rats. *J Endocrinol* 2006; **190**: 425-432

40 **Palomar-Morales M**, Morimoto S, Mendoza-Rodríguez CA, Cerbón MA. **The protective effect of testosterone on** streptozotocin-induced apoptosis in beta cells is sex specific. *Pancreas* 2010; **39**: 193-200

41 **Langley-Evans SC**, Gardner DS, Jackson AA. Association of disproportionate growth of fetal rats in late gestation with raised systolic blood pressure in later life. *J Reprod Fertil* 1996; **106**: 307-312

42 **Hales CN**, Barker DJ. The thrifty phenotype hypothesis. *Br Med Bull* 2001; **60**: 5-20

43 **Yajnik C**. Interactions of perturbations in intrauterine growth and growth during childhood on the risk of adult-onset disease. *Proc Nutr Soc* 2000; **59**: 257-265

44 **Cerf ME**, Chapman CS, Muller CJ, Louw J. Gestational high-fat programming impairs insulin release and reduces Pdx-1 and glucokinase immunoreactivity in neonatal Wistar rats. *Metabolism* 2009; **58**: 1787-1792

45 **Poston L**. Developmental programming and diabetes - The human experience and insight from animal models. *Best Pract Res Clin Endocrinol Metab* 2010; **24**: 541-552

46 **Ozaki T**, Nishina H, Hanson MA, Poston L. Dietary restriction in pregnant rats causes gender-related hypertension and vascular dysfunction in offspring. *J Physiol* 2001; **530**: 141-152

47 **Brown JL**, Spicer MT, Spicer LJ. Effect of high-fat diet on body composition and hormone responses to glucose tolerance tests. *Endocrine* 2002; **19**: 327-332

48 **Liang C**, DeCourcy K, Prater MR. High-saturated-fat diet induces gestational diabetes and placental vasculopathy in C57BL/6 mice. *Metabolism* 2010; **59**: 943-950

49 **Jansson N**, Pettersson J, Haafiz A, Ericsson A, Palmberg I, Tranberg M, Ganapathy V, Powell TL, Jansson T. Down-regulation of placental transport of amino acids precedes the development of intrauterine growth restriction in rats fed a low protein diet. *J Physiol* 2006; **576**: 935-946

50 **Rees WD**, Hay SM, Buchan V, Antipatis C, Palmer RM. The effects of maternal protein restriction on the growth of the rat fetus and its amino acid supply. *Br J Nutr* 1999; **81**: 243-250

51 **Dumortier O**, Blondeau B, Duvillié B, Reusens B, Bréant B, Remacle C. Different mechanisms operating during different critical time-windows reduce rat fetal beta cell mass due to a maternal low-protein or low-energy diet. *Diabetologia* 2007; **50**: 2495-2503

52 **Ericsson A**, Säljö K, Sjöstrand E, Jansson N, Prasad PD, Powell TL, Jansson T. Brief hyperglycaemia in the early pregnant rat increases fetal weight at term by stimulating placental growth and affecting placental nutrient transport. *J Physiol* 2007; **581**: 1323-1332

53 **Breton C**, Lukaszewski MA, Risold PY, Enache M, Guillemot J, Rivière G, Delahaye F, Lesage J, Dutriez-Casteloot I, Laborie C, Vieau D. Maternal prenatal undernutrition alters the response of POMC neurons to energy status variation in adult male rat offspring. *Am J Physiol Endocrinol Metab* 2009; **296**: E462-E472

54 **Eriksson UJ**, Bone AJ, Turnbull DM, Baird JD. Timed interruption of insulin therapy in diabetic BB/E rat pregnancy: effect on maternal metabolism and fetal outcome. *Acta Endocrinol* (Copenh) 1989; **120**: 800-810

55 **Bevier WC**, Jovanovic-Peterson L, Formby B, Peterson CM. Maternal hyperglycemia is not the only cause of macrosomia: lessons learned from the nonobese diabetic mouse. *Am J Perinatol* 1994; **11**: 51-56

56 **Yamashita H**, Shao J, Qiao L, Pagliassotti M, Friedman JE. Effect of spontaneous gestational diabetes on fetal and postnatal hepatic insulin resistance in Lepr(db/+) mice. *Pediatr Res* 2003; **53**: 411-418

57 **Ling ZC**, Efendic S, Wibom R, Abdel-Halim SM, Ostenson CG, Landau BR, Khan A. Glucose metabolism in Goto-Kakizaki rat islets. *Endocrinology* 1998; **139**: 2670-2675

58 **Galli J**, Li LS, Glaser A, Ostenson CG, Jiao H, Fakhrai-Rad H, Jacob HJ, Lander ES, Luthman H. Genetic analysis of non-insulin dependent diabetes mellitus in the GK rat. *Nat Genet* 1996; **12**: 31-37

59 **Gicquel C**, Le Bouc Y. Hormonal regulation of fetal growth. *Horm Res* 2006; **65** Suppl 3: 28-33

60 **Hiden U**, Maier A, Bilban M, Ghaffari-Tabrizi N, Wadsack C, Lang I, Dohr G, Desoye G. Insulin control of placental gene expression shifts from mother to foetus over the course of pregnancy. *Diabetologia* 2006; **49**: 123-131

61 **Hayati AR**, Cheah FC, Tan AE, Tan GC. **Insulin-like growth** factor-1 receptor expression in the placentae of diabetic and normal pregnancies. *Early Hum Dev* 2007; **83**: 41-46

62 **Yan-Jun L**, Tsushima T, Minei S, Sanaka M, Nagashima T, Yanagisawa K, Omori Y. Insulin-like growth factors (IGFs) and IGF-binding proteins (IGFBP-1, -2 and -3) in diabetic pregnancy: relationship to macrosomia. *Endocr J* 1996; **43**: 221-231

63 **Liu YJ**, Tsushima T, Onoda N, Minei S, Sanaka M, Nagashima T, Yanagisawa K, Omori Y. Expression of messenger RNA of insulin-like growth factors (IGFs) and IGF binding proteins (IGFBP1-6) in placenta of normal and diabetic pregnancy. *Endocr J* 1996; **43** Suppl: S89-S91

64 **Nelson SM**, Freeman DJ, Sattar N, Lindsay RS. Role of adiponectin in matching of fetal and placental weight in mothers with type 1 diabetes. *Diabetes Care* 2008; **31**: 1123-1125

65 **Hauguel-de Mouzon S**, Lepercq J, Catalano P. The known and unknown of leptin in pregnancy. *Am J Obstet Gynecol* 2006; **194**: 1537-1545

66 **Madani S**, De Girolamo S, Muñoz DM, Li RK, Sweeney G. Direct effects of leptin on size and extracellular matrix components of human pediatric ventricular myocytes. *Cardiovasc Res* 2006; **69**: 716-725

67 **Hiden U**, Desoye G. The placenta in diabetes in pregnancy. In: McCance DR, Maresh M, Sacks DA, editors. A practical manual of Diabetes in pregnancy. 1st ed. Oxford: Wiley-Blackwell, 2010: 26-33

S- Editor Wu X **L- Editor** Roemmele A **E- Editor** Zheng XM

World Journal of Diabetes

Online Submissions: http://www.wjgnet.com/1948-9358office
wjd@wjgnet.com
doi:10.4239/wjd.v2.i11.204

World J Diabetes 2011 November 15; 2(11): 204-210
ISSN 1948-9358 (online)

ORIGINAL ARTICLE

Biochemical and cellular evidence of the benefit of a combination of cerium oxide nanoparticles and selenium to diabetic rats

Nazila Pourkhalili, Asieh Hosseini, Amir Nili-Ahmadabadi, Shokoufeh Hassani, Mohsen Pakzad, Maryam Baeeri, Azadeh Mohammadirad, Mohammad Abdollahi

Nazila Pourkhalili, Amir Nili-Ahmadabadi, Shokoufeh Hassani, Mohsen Pakzad, Maryam Baeeri, Azadeh Mohammadirad, Mohammad Abdollahi, Faculty of Pharmacy, Pharmaceutical Sciences Research Center, Tehran University of Medical sciences, Tehran 1417614411, Iran

Asieh Hosseini, Razi Institute for Drug Research, Faculty of Medicine, Tehran University of Medical Sciences, Tehran 1417614411, Iran

Author contributions: Pourkhalili N researched the literature and carried out *in vivo* and *in vitro* parts of the work and prepared the results; Hosseini A searched the literature, checked results and drafted the manuscript; Nili-Ahmadabadi A performed *in vivo* and *in vitro* parts of the study; Hassani S helped in analyzing some of the biochemical parameters; Pakzad M assisted with the *in vivo* part of the study; Baeeri M carried out the biochemical analysis; Mohammadirad A compiled the literature bibliography, wrote the project and performed statistical analysis; Abdollahi M conceived the study, supervised/reviewed the entire study and edited the manuscript.

Supported by a grant from Tehran University of Medical Science, No. 11250 (in part)

Correspondence to: Mohammad Abdollahi, Professor, Faculty of Pharmacy, Pharmaceutical Sciences Research Center; Endocrinology and Metabolism Research Institute, Tehran University of Medical sciences, Tehran 1417614411, Iran. mohammad@tums.ac.ir

Telephone: +98-21-66959104 Fax: +98-21-66959104

Received: August 24, 2011 Revised: October 26, 2011
Accepted: October 31, 2011
Published online: November 15, 2011

Abstract

AIM: To study the combinative effects of nanocerium and selenium in a murine model of diabetes.

METHODS: Cerium oxide (CeO_2) nanoparticles (60 mg/kg per day) and sodium selenite (5 μmol/kg per day) alone or in combination, or the metal form of CeO_2 (60 mg/kg) were administered for 2 wk by intraperitoneal injection to streptozotocin-induced diabetic rats. At the end of treatment blood was collected, liver tissue dissected and then oxidative stress markers, extent of energy depletion and lipid profile were evaluated.

RESULTS: Antioxidant enzymes and high density lipoprotein decreased whereas oxidative stress, adenosine diphosphate/adenosine triphospahte levels, cholesterol, triglyceride and low density lipoprotein increased on induction of diabetes. All were improved by a combination of nanocerium and sodium selenite. There was a relative amelioration by CeO_2 nanoparticles or sodium selenite alone, but the metal form of CeO_2 showed no significant improvement.

CONCLUSION: The combination of nanocerium and sodium selenite is more effective than either alone in improving diabetes-induced oxidative stress.

Key words: Diabetes; Oxidative stress; Cerium oxide nanoparticles; Sodium selenite; Nanotoxicology; Nanotechnology

Peer reviewer: Khaled Abdul-Aziz Ahmed, Dr., Department of Medical Sciences, Ibb University, PO Box 70627, Ibb, Yemen

Pourkhalili N, Hosseini A, Nili-Ahmadabadi A, Hassani S, Pakzad M, Baeeri M, Mohammadirad A, Abdollahi M. Biochemical and cellular evidence of the benefit of a combination of cerium oxide nanoparticles and selenium to diabetic rats. *World J Diabetes* 2011; 2(11): 204-210 Available from: URL: http://www.wjgnet.com/1948-9358/full/v2/i11/204.htm DOI: http://dx.doi.org/10.4239/wjd.v2.i11.204

INTRODUCTION

Diabetes mellitus (DM) is an endocrine-metabolic disorder of increasing occurrence and clinical relevance, contributing to high morbidity and mortality rates. DM is increasing in the world due to population ageing, urbanization and obesity. With regard to the growing incidence, the study of the physiological roots of DM becomes important for the emergence of novel therapeutic procedures.

Increased oxidative stress is an important contributor to the development and progression of diabetes and its complications. Diabetes usually occurs with increased production of free radicals or impaired antioxidant defense[1]. Under diabetic conditions, glucose is prone to oxidation resulting in the generation of hydrogen peroxide and reactive intermediates such as the hydroxyl radical, the most reactive and toxic of free radicals[2].

The liver is the main organ involved in detoxifying free radicals and thus oxidative stress in liver happens in the early stages of diabetes. Strategies to reduce the formation of oxidative stress are important in the treatment of DM[3]. Cerium oxide (CeO_2) nanoparticles were thought to increase antioxidant power due to their catalytic effect in stimulating superoxide dismutase (SOD) activity and detoxifying free radicals by remaining active in tissues for extended periods through moving spontaneously between the oxidized and reduced state[4].

Selenium is an essential trace element that possesses a potent antioxidant effect in dysfunctions seen in diabetes[5].

Thus, given this knowledge of the antioxidant potential of CeO_2 nanoparticles and sodium selenite, and the pathophysiology of diabetes, the present study was aimed at evaluating the effects of these compounds, when used alone or in combination, on murine diabetes.

MATERIALS AND METHODS

Materials

Adenosine diphosphate (ADP) sodium salt, adenosine triphospahte (ATP) disodium salt, Tris base, 1,1,3,3-tetraethoxypropane (MDA), 5,5'dithiobis-2-nitro benzoic acid (DTNB), methanol [high performance liquid chromatography (HPLC)-grade], trichloroacetic acid (TCA), potassium hydroxide, diethyl ether, tetrabutylammonium hydroxide, n-butanol, 2-thiobarbituric acid (TBA), KH_2PO_4 (analytical grade), 2,4,6-tripyridyl-s-triazine (TPTZ), sodium selenite, H_2O_2, phosphate buffer solution (PBS) from Merck (Tehran), 2,7-dichlorodihydrofluorescein diacetate from Sigma-Aldrich (Taufkirchen, Germany), CeO_2 nanoparticles from Navarrean Nenoproducts Technology (Spain), streptozotocine (STZ) from Pharmacia and Upjohn (United States), SOD kit from Randox (United Kingdom) and a SUPELCOSIL™ LC-18-T HPLC column from SUPELCO (United Kingdom), commercial kits for cholesterol, triglyceride, low density lipoprotein (LDL), high density lipoprotein (HDL) from Parsazmoon (Tehran) and ketamine/xylazin from a local pharmacy were used in this study.

Animals and experimental design and sampling

Male Wistar rats (180-200 g) were used. *In vivo* studies were performed according to ethical guidelines on the use of animals in research and the protocol was approved by the TUMS/PSRC review board. Experimental diabetes was induced in fasted rats by a single intraperitoneal (ip) injection of streptozotocin at a dose of 75 mg/kg in 0.1 mol/L citrate buffer at pH 4.5. Within 1 wk of injection, rats showing blood glucose values above 300 mg/dL were included in the study.

Animals were randomly divided into six groups with six rats in each group. Group 1 rats (normal control group) were injected intraperitoneally with normal saline (NS) for 2 wk. Group 2 rats (diabetes control group) received a single ip injection of STZ (75 mg/kg) and NS for 2 wk. Group 3 rats were injected with treatment STZ (75 mg/kg) and the metal form of CeO_2 (60 mg/kg). In Group 4 CeO_2 nanoparticles (60 mg/kg) were used instead of the metal form of CeO_2. In Groups 5 and 6, a single dose of STZ (75 mg/kg) was used in addition to sodium selenite (5 μmol/kg per day) (Group 5) or a combination of sodium selenite (5 μmol/kg per day) and CeO_2 nanoparticles (60 mg/kg) (Group 6).

For sample preparation, animals were anesthetized with an intramuscular (im) injection of ketamine (4 mg/100 g) and xylazine (1 mg/100 g) mixture. Blood was collected from the heart into a heparinized syringe. Blood samples were centrifuged at 1200 g for 10 min at 4℃ and plasma was frozen at -80℃ until use. Liver tissue was dissected and stored immediately on ice and then 100 mg of liver was homogenized in PBS (50 mmol/L, pH 7), then centrifuged at 30000 g for 30 min at 4℃. The supernatant was collected and stored at -80℃. Extra liver tissues were frozen quickly in liquid nitrogen for further analysis.

Biochemical assays

At the end of treatment, blood glucose levels were measured using a glucometer and the animal's weight was determined using an animal balance.

Total antioxidant capacity: Reduction of Fe^{3+} to Fe^{2+} by the biological sample is an indicator of antioxidant capacity. The complex between Fe^{2+} and TPTZ produces a blue color with a maximum absorbance at 593 nm as previously described[6].

Total thiol molecules: Total thiol group was determined using DTNB as the reagent. One hundred microliters of the sample was mixed with 0.1 mol/L 1500 μL phosphate buffer pH 7.4 and 400 μL of 2 mol/L DTNB. After incubation at 37℃ for 30 min, absorbance of the samples was measured against a blank at 412 nm.

Lipid peroxidation: Thiobarbituric acid-reactive substances were measured using 1,1',3,3'- tetraethoxypropane as a standard and from a standard curve of TBA adduct formation[6].

Catalase: The activity of catalase (CAT) was measured

Table 1 Effects of various treatments on body weight and liver weight/body weight (mean ± SE)

Animal groups	Body weight index		Liver weight index	
	Initial (g)	Final (g)	LW (g)	BW/LW
Control	187.25 ± 3.68	207.5 ± 3.22	7.54 ± 0.23	25.86 ± 1.18
Diabetic control	185.75 ± 3.94	172.6 ± 1.76[a]	10.02 ± 0.30[c]	16.5 ± 0.78[c]
Cerium oxide	192.25 ± 6.14	185.6 ± 4.70[f]	9.77 ± 0.42[b]	19.59 ± 0.86[b,f]
Nanocerium oxide	189.00 ± 3.71	202.2 ± 2.01[d]	8.35 ± 0.12[d]	24.15 ± 0.49[e]
Sodium selenite	187.75 ± 5.80	193.3 ± 6.38	8.69 ± 0.44	22.31 ± 1.00[d]
Nanocerium oxide + Sodium selenite	195.20 ± 3.00	214.8 ± 8.77[e]	9.73 ± 0.38[b]	24.11 ± 1.04[e]

[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ *vs* control group; [d]$P < 0.01$, [e]$P < 0.01$ *vs* diabetic control group; [f]$P < 0.05$ vs Nanocerium oxide + Sodium selenite group. LW: Liver weight; BW: Body weight.

by observing the initial rate of hydrogen peroxide disappearance in a spectrophotometer at 240 nm. Results are reported as a constant rate per second per liter plasma and as micromoles of formaldehyde produced per mg of protein[7].

SOD stimulation: According to the kit protocol, xanthine and xanthine oxidase generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) and create a red formazon dye. The SOD activity is measured by the degree of inhibition of this reaction. There is 50% inhibition of the rate of reduction of INT by one unit of SOD. Data are shown as unit/mg of protein.

Reactive oxygen species: The activity of reactive oxygen species (ROS) was determined by use of DCF-DA, which is converted into highly fluorescent DCF by cellular peroxides. The sample was divided into two equal parts. In one fraction, 40 μL of 1.25 mmol/L DCF-DA in methanol was added for ROS determination. In the other fraction 40 μL of methanol was added, as a control for tissue auto-fluorescence. All samples were incubated for 15 min in a 37℃ water-bath.. Fluorescence was measured at 488 nm excitation and 525 nm emission, using a fluorescence plate reader as described previously[8]. Results were expressed as nmol/min/mg of protein.

Cholesterol, triglyceride and lipoproteins: Aliquots of serum were taken for determination of total cholesterol by the enzymatic colorimetric assay method and triglycerides determined by enzymatic glycerol phosphate oxidase/peroxidase method, Autoanalyzer and Elitech kit were used. LDL was precipitated by adding phosphotungstic acid and magnesium ions to the serum. Centrifugation left only the HDL in the supernatant.

ADP/ATP: The frozen liver was removed and quickly homogenized (4℃) in 1 mL of ice-cold 6% TCA. The homogenate was centrifuged at 12000 g for 10 min at 4℃ and the supernatant was neutralized to a pH of 6.5 with 4 mol/L KOH. After filtering through a Millipore filter, the neutralized extract was used to determine ATP and ADP concentration (μg/mL per mg of tissue) by ion-pair HPLC. Standard curves were created using standard solutions of ATP and ADP and then samples were tested to measure energy changes as an ADP/ATP ratio[9].

Statistical analysis: All values were reported as a mean ± SE. Statistical analysis of data was carried out by analysis of variance followed by Newman-Keuls and Stat Direct version 2.7.7. It should be noted that, P values less than 0.05 were considered statistically significant.

RESULTS

Animal's weight and blood glucose

The weight of diabetic rats decreased significantly compared to control rats ($P < 0.05$). Blood glucose in diabetic rats significantly increased compared with control rats ($P < 0.001$). After use of sodium selenite and CeO_2 nanoparticles combined and CeO_2 nanoparticles alone, a significant increase in animal weight ($P < 0.01$ and $P < 0.05$, respectively) was observed compared to diabetic rats. Also, a significant decrease in blood glucose ($P < 0.05$) was shown only by the combination of sodium selenite and CeO_2 nanoparticles, when compared to diabetic rats. No significant changes in animal weight were observed when using sodium selenite and the metal form of CeO_2 (Table 1 and Figure 1).

The liver weight/body weight index in diabetic rats significantly decreased ($P < 0.001$) compared with control animals. Administration of sodium selenite, CeO_2 nanoparticles and a combination of sodium selenite and CeO_2 nanoparticles led to an increase in this index ($P < 0.05$, $P < 0.01$, respectively) as compared to diabetic rats (Table 1).

Biomarkers of oxidative stress

A significant increase in lipid peroxidation (LPO) ($P < 0.001$) and a decrease in total antioxidant capacity (TAC) and total thiol molecules (TTM) ($P < 0.001$ and $P < 0.01$, respectively) in the diabetic group were recorded in comparison to controls in both plasma and liver (Table 2). Administration of sodium selenite, CeO_2 nanoparticles and a combination of sodium selenite and CeO_2 nanoparticles decreased LPO ($P < 0.05$ in plasma and liver, $P < 0.01$ in plasma, $P < 0.05$ in liver, $P < 0.001$ in plasma and liver, respectively) as compared to the diabetic group. The metal form of CeO_2 caused no significant changes. A significant

Table 2 Effects of various treatments on oxidative stress biomarkers in plasma and liver (mean ± SE)

Groups	LPO		TAC		TTM	
	Plasma (nmol/mL)	Liver (nmol/mg protein)	Plasma (μmol/mL)	Liver (nmol/mg protein)	Plasma (mmol/L)	Liver (nmol/mg protein)
Control	2.08 ± 0.36	2.16 ± 0.23	528.62 ± 7.45	5.47 ± 0.34	0.512 ± 0.026	10.02 ± 0.36
Diabetic control	6.97 ± 0.33[b]	5.37 ± 0.34[b]	224.20 ± 13.43[b]	1.45 ± 0.19[b]	0.376 ± 0.024[a]	7.91 ± 0.30[a]
Cerium oxide	6.85 ± 0.42[b,h]	5.31 ± 0.31[b,h]	231.43 ± 5.68[b,h]	1.73 ± 0.23[b,h]	0.360 ± 0.016[a,g]	6.67 ± 0.40[b,h]
Nanocerium oxide	4.92 ± 0.42[b,d,f]	4.12 ± 0.22[b,c,f]	272.33 ± 10.49[b,h]	3.13 ± 0.29[b,d,h]	0.379 ± 0.010[a,f]	7.10 ± 0.25[b,h]
Sodium selenite	5.14 ± 0.33[b,c,g]	3.95 ± 0.22[a,c,f]	257.46 ± 12.28[b,h]	2.98 ± 0.17[b,d,h]	0.480 ± 0.025[c,f]	9.32 ± 0.32[c]
Nanocerium oxide + Sodium selenite	3.19 ± 0.30[e]	2.60 ± 0.26[e]	493.46 ± 7.45[e]	4.97 ± 0.28[e]	0.500 ± 0.024[d]	9.83 ± 0.30[d]

[a]$P < 0.01$, [b]$P < 0.001$ *vs* control group; [c]$P < 0.01$, [d]$P < 0.01$, [e]$P < 0.001$ *vs* diabetic control group; [f]$P < 0.05$, [g]$P < 0.01$, [h]$P < 0.001$ *vs* Nanocerium oxide + Sodium selenite group. LPO: Lipid peroxidation; TAC: Total antioxidant capacity; TTM: Total thiol molecules.

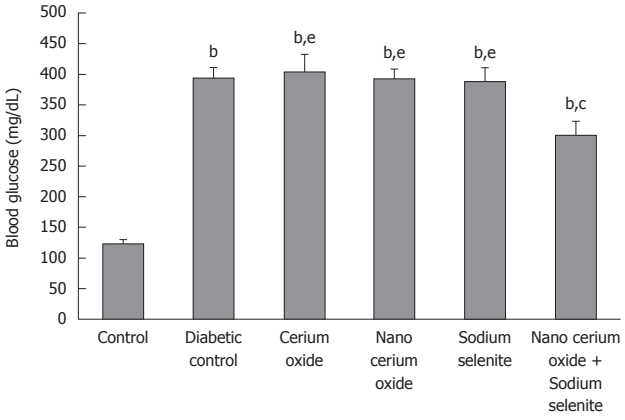


Figure 1 Effects of various treatments on blood glucose in diabetic rats. Data are mean ± SE of six animals. [b]$P < 0.001$ *vs* control group; [c]$P < 0.05$ *vs* diabetic control group; [e]$P < 0.05$ *vs* Nanocerium oxide + Sodium selenite group.

increase of TAC ($P < 0.001$) was observed by the sodium selenite/CeO_2 nanoparticle combination in plasma and liver. Use of sodium selenite and CeO_2 nanoparticles in liver increased TAC (both $P < 0.01$) in comparison to the diabetic group. No significant changes were produced by the metal form of CeO_2, sodium selenite, and CeO_2 nanoparticles in plasma or by the metal form of CeO_2 in liver. The level of TTM after use of sodium selenite and a combination of sodium selenite and CeO_2 nanoparticles showed a significant increase ($P < 0.05$ and $P < 0.01$, respectively) compared to the diabetic group in both plasma and liver. No significant changes were detected by administration of CeO_2 nanoparticles and the metal form of CeO_2 (Table 2).

CAT and SOD

In the diabetic group there was a significant decrease in CAT activity in both plasma and liver ($P < 0.001$) compared to control rats. However, administration of CeO_2 nanoparticles, sodium selenite, and combination of sodium selenite and CeO_2 nanoparticles caused a significant increase in this marker ($P < 0.01$, $P < 0.05$ and $P < 0.001$, respectively). No significant changes were observed after administration of the metal form of CeO_2 (Table 3). SOD activity in both plasma and liver of diabetic rats significantly decreased as compared with controls ($P < 0.01$ and $P < 0.001$, respectively). After use of CeO_2 nanoparticles, and a combination of sodium selenite and CeO_2 nanoparticles, a significant increase in plasma SOD activity ($P < 0.05$) was observed compared to diabetic rats. No significant changes were observed when using sodium selenite and the metal form of CeO_2. However, there was a significant increase in SOD activity in liver ($P < 0.01$) compared with diabetic rats only by combination of sodium selenite and CeO_2 nanoparticles (Table 3).

ROS

In the diabetic rats, hepatic ROS significantly increased ($P < 0.001$), but returned to almost normal, after use of sodium selenite, CeO_2 nanoparticles and a combination of sodium selenite and CeO_2 nanoparticles ($P < 0.05$ and $P < 0.001$, respectively). No significant changes were observed after administration of the metal form of CeO_2 (Figure 2).

Cholesterol, triglyceride and lipoproteins

Total plasma cholesterol, triglycerides and LDL were significantly elevated ($P < 0.01$, $P < 0.001$ and $P < 0.01$, respectively) in diabetic rats as compared to the control group. Similarly, HDL was significantly reduced ($P < 0.01$) in diabetic rats as compared with the control group (Table 4). No significant changes were observed in plasma cholesterol by use of different treatments. Triglycerides were reduced ($P < 0.05$ and $P < 0.01$, respectively) by use of CeO_2 nanoparticles, sodium selenite and a combination of sodium selenite and CeO_2 nanoparticles as compared to the diabetic group but there was no significant change when using the metal form of CeO_2. Administration of sodium selenite and a combination of sodium selenite and CeO_2 nanoparticles significantly reduced ($P < 0.05$) plasma LDL compared to the diabetic group. The nano and metal forms of of CeO_2 did not cause significant change.

Plasma HDL was significantly improved ($P < 0.05$) by all treatments except for the metal form of CeO_2 (Table 4).

ADP/ATP

As observed in Figure 2, a significant increase in the liver

Table 3 Effects of various treatments on plasma, liver catalase and superoxide dismutase (mean ± SE)

Groups	CAT activity		SOD activity	
	Plasma (U/mL)	Liver (U/mg protein)	Plasma (U/mL)	Liver (U/mg protein)
Control	13.74 ± 0.26	76.25 ± 1.39	7.81 ± 3.98	16.22 ± 0.56
Diabetic control	5.39 ± 0.70^{c}	51.26 ± 1.67^{c}	6.30 ± 2.33^{b}	11.78 ± 0.91^{c}
Cerium oxide	6.50 ± 0.74^{c,i}	52.20 ± 2.70^{c,i}	6.18 ± 3.13^{b,g}	10.02 ± 0.57^{c,i}
Nanocerium oxide	9.05 ± 0.51^{c,e,h}	66.38 ± 3.81^{e}	7.45 ± 1.99^{d}	12.37 ± 0.54^{b,g}
Sodium selenite	8.31 ± 0.73^{c,d,i}	63.09 ± 2.13^{b,d,g}	6.50 ± 1.51^{a}	11.78 ± 0.56^{c,h}
Nanocerium oxide + Sodium selenite	12.86 ± 0.44^{f}	72.75 ± 1.74^{f}	7.68 ± 2.55^{d}	15.55 ± 0.78^{e}

$^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ *vs* control group; $^{d}P < 0.01$, $^{e}P < 0.01$, $^{f}P < 0.001$ *vs* diabetic control group; $^{g}P < 0.05$, $^{h}P < 0.01$, $^{i}P < 0.001$ *vs* Nanocerium oxide + Sodium selenite group. CAT: Catalase; SOD: Superoxide dismutase.

Table 4 Effects of various treatments on plasma cholesterol, triglyceride and lipoproteins (mean ± SE)

Animal Groups	Cholestrol (mg/dL)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
Control	69.41 ± 7.02	70.66 ± 6.66	41.28 ± 2.27	36.37 ± 1.91
Diabetic control	110.20 ± 5.09^{b}	166.09 ± 8.53^{c}	30.14 ± 1.83^{b}	51.42 ± 2.80^{b}
Cerium oxide	109.98 ± 5.53^{c}	166.05 ± 10.03^{c,f}	29.00 ± 1.95^{c,f}	60.14 ± 1.62^{c,g}
Nanocerium oxide	107.02 ± 1.48^{b}	123.37 ± 8.72^{b,d}	38.42 ± 1.46^{d}	49.00 ± 2.80^{a}
Sodium selenite	102.22 ± 6.77^{b}	123.25 ± 6.43^{b,d}	38.00 ± 1.43^{d}	40.66 ± 2.66^{d}
Nanocerium oxide + Sodium selenite	106.15 ± 5.79^{b}	113.10 ± 8.17^{a,e}	39.60 ± 2.13^{d}	39.14 ± 2.79^{d}

$^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ *vs* control group; $^{d}P < 0.01$, $^{e}P < 0.01$ *vs* diabetic control group; $^{f}P < 0.01$, $^{g}P < 0.001$ *vs* Nanocerium oxide + Sodium selenite group. HDL: High density lipoprotein; LDL: Low density lipoprotein.

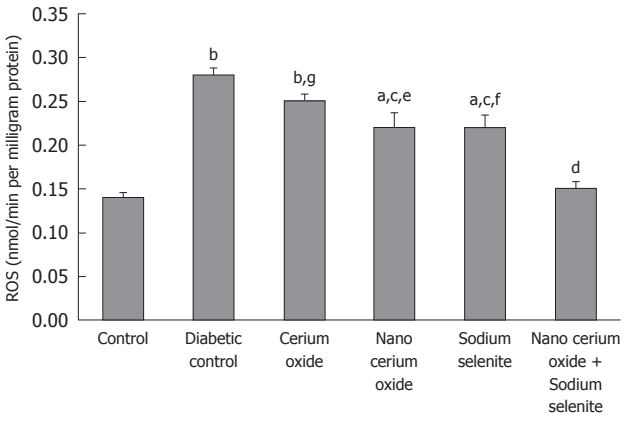


Figure 2 Effects of various treatments on liver reactive oxygen species in diabetic rats. Data are mean ± SE of six animals. $^{a}P < 0.01$, $^{b}P < 0.001$ *vs* control group; $^{c}P < 0.05$, $^{d}P < 0.001$ *vs* diabetic control group; $^{e}P < 0.05$, $^{f}P < 0.01$, $^{g}P < 0.001$ *vs* Nanocerium oxide + Sodium selenite group. ROS: Reactive oxygen species.

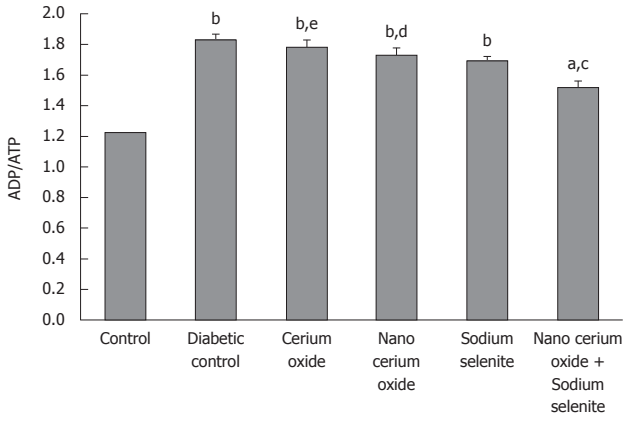


Figure 3 Effects of various treatments on adenosine diphosphate/adenosine triphospahte in diabetic rats. Data are mean ± SE of six animals. $^{a}P < 0.01$, $^{b}P < 0.001$ *vs* control group; $^{c}P < 0.01$ *vs* diabetic control group; $^{d}P < 0.05$, $^{e}P < 0.01$ *vs* Nanocerium oxide + Sodium selenite group. ADP/ATP: Adenosine diphosphate/adenosine triphospahte.

ADP/ATP ratio in diabetic rats is evident in comparison to the control group ($P < 0.001$). The administration of a combination of sodium selenite and CeO_2 nanoparticles reduced the ADP/ATP ratio in comparison to the diabetic group ($P < 0.01$); sodium selenite, CeO_2 nanoparticles, or metal form of CeO_2 used separately caused no significant change (Figure 3).

DISCUSSION

The present study indicates a significant improvement in biomarkers of diabetes including oxidative stress, energy compensation (ADP/ATP) and lipid profile by using a combination of sodium selenite and CeO_2 nanoparticles. It is mentioned that a relative improvement in these biomarkers was shown by CeO_2 nanoparticles and sodium selenite used separately, but no significant changes were found when the metal form of CeO_2 was used. This is the first report on the benefit of a CeO_2 nanoparticles/sodium selenite combination in diabetes treatment. The unique structure of CeO_2 nanoparticles supports a potential role as a biological free radical scavenger or antioxidant. This metal oxide is characterized by monodisperse particles that are single crystals with few twin

boundaries[10] and expanded lattice parameter[11] making this compound nonstoichiometric. Moreover, the cerium atom is characterized by dual oxidation state potential and oxygen vacancies[12]. This property is responsible for the free radical scavenging activity of CeO_2 that makes it beneficial in diabetes. Other than the antioxidant effect of CeO_2, it has many sites for catalysis that makes it more active and resident in a living cell for an extended period of time. In addition, CeO_2 nanoparticles stimulate SOD activity[13,14].

On the other hand, selenium is an essential trace element possessing cardioprotective, antiproliferative and chemopreventive effects[14-16]. It is also a potent antioxidant for dysfunctions seen in diabetes[5] and colitis[17].

In fact, a clear link between oxidative stress and diabetes exists where liver is the main organ involved because the liver is rich in mitochondria to perform metabolic functions. Liver plays an important role in glucose metabolism, and in a chronic hyperglycemic state, liver oxidative stress is considered a relevant process. Oxidative stress induced by hyperglycemia leads to liver cell damage because liver is subject to ROS-mediated injury in diabetes. In the present study, a significant increase of oxidative stress biomarkers, ROS and a reduction in antioxidant enzymes in plasma and liver was observed and this has support from previous reports, as mentioned above.

On the other hand, ROS plays an important role in the regulation of hepatic glucose production. The antidiabetic drugs which act through inhibition of hepatic gluconeogenesis produce concurrent antioxidant effects beneficial in the treatment of diabetes[18]. Thus, the antioxidant potential of CeO_2 nanoparticles and selenium might be a mechanism for their glucose lowering effect and inhibition of glycogenolysis.

In diabetes, increased ROS coupled with depolarization of the inner mitochondrial membrane reduces ATP. Considering the large number of mitochondria present in liver, reduction in the ATP of liver in diabetes conditions seems rational[19] and explains the present reduction of liver ADP/ATP in diabetic rats.

The present findings also showed a significant increase in triglycerides, LDL, cholesterol and a reduction in the HDL of diabetic animals which were expected. One of the most important complications of diabetes is atherosclerosis and coronary heart disease, the result of abnormal lipid metabolism, hyperglycemia may promote LPO of LDL resulting in the generation of free radicals. The lipid profile of diabetes is characterized by low levels of HDL, elevated LDL and TG levels[20].

In conclusion, the combination of CeO_2 nanoparticles/sodium selenite shows the best anti-oxidative effects beneficial in experimental diabetes. Therefore, this combination should be followed for further tests and clinical trials.

COMMENTS

Background

Diabetes mellitus (DM) is an endocrine-metabolic disorder of increasing occurrence and clinical relevance, contributing to high morbidity and mortality rates. DM is increasing in the world by population ageing, urbanization and obesity. The study of the physiological routes of DM is important for the development of novel therapeutic procedures for this increasingly common disease.

Research frontiers

Increased oxidative stress is an important contributor to the development and progression of diabetes and its complications. Strategies to reduce the formation of oxidative stress are important in the treatment of DM. It seems that cerium oxide (CeO_2) nanoparticles and sodium selenite, as two powerful antioxidants, are suitable for this purpose. The present study was aimed to evaluate the effects of these compounds on murine diabetes when used alone or in combination.

Innovations and breakthroughs

The present study indicates a significant improvement in biomarkers of diabetes including oxidative stress, energy compensation [adenosine diphosphate/adenosine triphospahte (ADP/ATP)] and lipid profile by using a combination of sodium selenite and CeO_2 nanoparticles. It is mentioned that a relative improvement in these biomarkers was shown by CeO_2 nanoparticles and sodium selenite when used alone but no significant change was found when the metal form of CeO_2 was used. This is the first report on the benefit of a CeO_2 nanoparticles/sodium selenite combination in diabetes.

Applications

DM is increasing in the world due to population ageing, urbanization and obesity and causes high morbidity and mortality rates. We suggest a combination of nanocerium and sodium selenite for the treatment of diabetes and its complications.

Terminology

CeO_2 nanoparticle - a powerful antioxidant with free radical scavenging properties. Selenium - an essential trace element that possesses a potent antioxidant effect.

Peer review

The major finding of the study is that a combination of sodium selenite and CeO_2 nanoparticles indicates a significant improvement in biomarkers of diabetes including oxidative stress, energy compensation (ADP/ATP) and lipid profile. In fact, this combination shows the most beneficial anti-oxidative effects in experimental diabetes. Therefore, this combination should be followed for further tests and clinical trials.

REFERENCES

1 **Rahimi R**, Nikfar S, Larijani B, Abdollahi M. A review on the role of antioxidants in the management of diabetes and its complications. *Biomed Pharmacother* 2005; **59**: 365-373

2 **Kajbaf F**, Mojtahedzadeh M, Abdollahi M. Mechanisms underlying stress-induced hyperglycemia in critically ill patients. *Therapy* 2007; **4**: 97-106

3 **Di Naso FC**, Simões Dias A, Porawski M, Marroni NA. Exogenous superoxide dismutase: action on liver oxidative stress in animals with streptozotocin-induced diabetes. *Exp Diabetes Res* 2011; **2011**: 754132

4 **Andresscu ES**, Leiter JC, Erlichman JS, inventors;Clarkson University, assignee. Method of neuroprotection from oxidant injury using metal oxide nanoparticles. United States patent US 2010/0098768 A1. 2010 Apr 22

5 **Ayaz M**, Celik HA, Aydin HH, Turan B. Sodium selenite protects against diabetes-induced alterations in the antioxidant defense system of the liver. *Diabetes Metab Res Rev* 2006; **22**: 295-299

6 **Astaneie F**, Afshari M, Mojtahedi A, Mostafalou S, Zamani MJ, Larijani B, Abdollahi M. Total antioxidant capacity and levels of epidermal growth factor and nitric oxide in blood and saliva of insulin-dependent diabetic patients. *Arch Med Res* 2005; **36**: 376-381

7 **Ranjbar A**, Ghahremani MH, Sharifzadeh M, Golestani A, Ghazi-Khansari M, Baeeri M, Abdollahi M. Protection by pentoxifylline of malathion-induced toxic stress and mitochondrial damage in rat brain. *Hum Exp Toxicol* 2010; **29**: 851-864

8 **Momtaz S**, Lall N, Hussein A, Ostad SN, Abdollahi M. In-

vestigation of the possible biological activities of a poisonous South African plant; Hyaenanche globosa (Euphorbiaceae). *Pharmacogn Mag* 2010; **6**: 34-41

9 **Hosseini A**, Sharifzadeh M, Rezayat SM, Hassanzadeh G, Hassani S, Baeeri M, Shetab-Bushehri V, Kuznetsov DA, Abdollahi M. Benefit of magnesium-25 carrying porphyrin-fullerene nanoparticles in experimental diabetic neuropathy. *Int J Nanomedicine* 2010; **5**: 517-523

10 **Zhang F**, Chan SW, Spanier JF, Apak F, Jin Q, Robinson RD, Herman IP. Cerium oxide nanoparticles: size-selective formation and structure analysis. *Appl Phys Lett* 2002; **80**: 127-129

11 **Perebeinos V**, Chan SW, Zhang F. 'Madelung model' prediction for dependence of lattice parameter on nanocrystal size. *Solid State Commun* 2002; **123**: 295-297

12 **Robinson RD**, Spanier JE, Zhang F, Chan SW, Herman IP. Visible thermal emission from sub-band-gap laser excited cerium dioxide particles. *J Appl Phys* 2002; **92**: 1936-1941

13 **Korsvik C**, Patil S, Seal S, Self WT. Superoxide dismutase mimetic properties exhibited by vacancy engineered ceria nanoparticles. *Chem Commun* (Camb) 2007; **10:** 1056-1058

14 **Celik HA**, Aydin HH, Deveci R, Terzioglu E, Karacali S, Saydam G, Akarca U, Batur Y. Biochemical and morphological characteristics of selenite-induced apoptosis in human hepatoma Hep G2 cells. *Biol Trace Elem Res* 2004; **99**: 27-40

15 **Venardos K**, Harrison G, Headrick J, Perkins A. Effects of dietary selenium on glutathione peroxidase and thioredoxin reductase activity and recovery from cardiac ischemia-reperfusion. *J Trace Elem Med Biol* 2004; **18**: 81-88

16 **Abdollahi M**, Rahmat-Jirdeh N, Soltaninejad K. Protection by selenium of lead-acetate-induced alterations on rat submandibular gland function. *Hum Exp Toxicol* 2001; **20**: 28-33

17 **Miroliaee AE**, Esmaily H, Vaziri-Bami A, Baeeri M, Shahverdi AR, Abdollahi M. Amelioration of experimental colitis by a novel nanoselenium-silymarin mixture. *Toxicol Mech Methods* 2011; **21**: 200-208

18 **Hoseini S**, Esmaily H, Mohammadirad A, Abdollahi M. Effects of Sildenafil a Phosphodiesterase 5 Inhibitor on Rat Liver Cell Key Enzymes of Gluconeogenesis and Glycogenolysis. *Int J Pharmacol* 2006; **2**: 280-285

19 **Rahimi R**, Abdollahi M. A review on the mechanisms involved in hyperglycemia induced by organophosphorus pesticides. *Pestic Biochem Phys* 2007; **88**: 115-121

20 **Vosough-Ghanbari S**, Rahimi R, Kharabaf S, Zeinali S, Mohammadirad A, Amini S, Yasa N, Salehnia A, Toliat T, Nikfar S, Larijani B, Abdollahi M. Effects of Satureja khuzestanica on Serum Glucose, Lipids and Markers of Oxidative Stress in Patients with Type 2 Diabetes Mellitus: A Double-Blind Randomized Controlled Trial. *Evid Based Complement Alternat Med* 2010; **7**: 465-470

S- Editor Wu X **L- Editor** Hughes D **E- Editor** Zheng XM

World Journal of Diabetes

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

World J Diabetes 2011 November 15; 2(11): I
ISSN 1948-9358 (online)

Acknowledgments to reviewers of *World Journal of Diabetes*

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

Christa Buechler, Dr., Department of Internal Medicine I, Regensburg University Hospital, Franz Josef Strauss Allee 11, Regensburg 93042, Germany

Motoaki Saito, Dr., Department of Mol Pharmacology, Tottori University, 86 Nishimachi, Yonago 683-8503, Japan

Pappachan M Joseph, Dr., Department of Medicine, Pariyaram Medical College, C/o Adv Nicholas Joseph, Court Road, Taliparamba, Kannur 670141, India

Rob Weijers, Dr., Teaching Hospital, Onze Lieve Vrouwe Gasthuis, Oosterpark 9, Amsterdam, 1090 HM, The Netherlands

David J Hill, Professor, Lawson Health Research Institute, 268 Grosvenor Street, London N6A4V2, Canada

KVS Hari Kumar, Dr., Department of Endocrinology, Command Hospital, Central Command, Lucknow Cantt, Lucknow 226002, India

Khaled Abdul-Aziz Ahmed, Dr., Department of Medical Sciences, Ibb University, PO Box 70627, Ibb, Yemen

James Edward Foley, Dr., Departemnt of Medical Affairs, Novartis Pharmaceuticals Corporation, East Hanover, NJ 07936-1080, United States

Goji Hasegawa, Dr., Department of Endocrinology and Metabolism, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan

World Journal of Diabetes

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

World J Diabetes 2011 November 15; 2(11): I
ISSN 1948-9358 (online)

Events Calendar 2011

January 14-15, 2011
AGA Clinical Congress of Gastroenterology and Hepatology: Best Practices in 2011 Miami
FL, United States

January 28, 2011
Diabetes UK and External Conferences
Diabetes Awareness Training
London, United Kingdom

January 28-29, 2011
9. Gastro Forum München
Munich, Germany

February 13-27, 2011
Gastroenterology: New Zealand CME Cruise Conference
Sydney, NSW, Australia

February 16-19, 2011
The 4th International Conference on Advance Technologies & Treatments for Diabetes
London, United Kingdom

February 24-26, 2011
2nd International Congress on Abdominal Obesity
Buenos Aires, Brazil

February 26-March 1, 2011
Canadian Digestive Diseases Week,
Westin Bayshore, Vancouver
British Columbia, Canada

February 28-March 1, 2011
Childhood & Adolescent Obesity: A Whole-system Strategic Approach
Abu Dhabi, United Arab Emirates

March 3-5, 2011
42nd Annual Topics in Internal Medicine
Gainesville, FL, United States

March 14-17, 2011
British Society of Gastroenterology Annual Meeting 2011, Birmingham
England, United Kingdom

March 17-20, 2011
Mayo Clinic Gastroenterology & Hepatology
Jacksonville, FL , United States

March 18, 2011
UC Davis Health Informatics: Change Management and Health Informatics, The Keys to Health Reform
Sacramento, CA, United States

March 25-27, 2011
MedicReS IC 2011 Good Medical Research
Istanbul, Turkey
March 28–30, 2011
The Second World Congress on Interventional Therapies for Type 2 Diabetes
New York, United States

April 25-27, 2011
The Second International Conference of the Saudi Society of Pediatric Gastroenterology, Hepatology & Nutrition
Riyadh, Saudi Arabia

May 7-10, 2011
Digestive Disease Week
Chicago, IL, United States

June 2-5, 2011
The 1st Asia Pacific Congress on Controversies to Consensus in Diabetes, Obesity and Hypertension
Shanghai, China

June 11-12, 2011
The International Digestive Disease Forum 2011
Hong Kong, China

June 22-25, 2011
ESMO Conference: 13th World Congress on Gastrointestinal Cancer
Barcelona, Spain

August 3-6, 2011
AADE 38th Annual Meeting
Las Vegas, United States
October 16-18, 2011
ISPAD Science School for Health Professionals
Miami, Unites States

October 19-22, 2011
ISPAD 36th Annual Meeting
Miami, United States

October 22-26, 2011
19th United European Gastroenterology Week
Stockholm, Sweden

October 26-29, 2011
CDA/CSEM Professional Conference and Annual Meetings
Toronto, Ontario, Canada

October 28-November 2, 2011
ACG Annual Scientific Meeting & Postgraduate Course
Washington, DC, United States

November 10-12, 2011
The Second International Diabetes & Obesity Forum
Istanbul, Turkey

World Journal of Diabetes

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

World J Diabetes 2011 November 15; 2(11): I-V
ISSN 1948-9358 (online)

INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION

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

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

Maximization of personal benefits

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

Aims and scope

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

Columns

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

Name of journal

World Journal of Diabetes

ISSN

ISSN 1948-9358 (online)

Indexing/abstracting

PubMed Central, PubMed, Digital Object Identifer, and Directory of Open Access Journals.

Published by

Baishideng Publishing Group Co., Limited

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the

authors except where indicated otherwise.

Biostatistical editing

Statisital review is performed after peer review. We invite an expert in Biomedical Statistics from to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement

In the interests of transparency and to help reviewersassess any potential bias, *WJD* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent

Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights

When reporting the results from experiments, authors should follow the highest standards and the trial should comform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the International Committee of Medical Journal Editors to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www. clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

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

Online submissions

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

MANUSCRIPT PREPARATION

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

Title page

Title: Title should be less than 12 words.

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

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

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

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

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

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

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

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

Abstract

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

An informative, structured abstracts of no more than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…; MATERIALS AND METHODS (no more than 140 words); RESULTS (no more than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

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

Text

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

Illustrations

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

Tables

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

Notes in tables and illustrations

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

Acknowledgments

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

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated

with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that..."

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

PMID and DOI

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

Style for journal references

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

Style for book references

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

Format

Journals

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

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

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

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

In press

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

Organization as author

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

Both personal authors and an organization as author

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

No author given

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

Volume with supplement

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

Issue with no volume

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

No volume or issue

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

Books

Personal author(s)

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

Chapter in a book (list all authors)

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

Author(s) and editor(s)

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

Conference proceedings

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

Conference paper

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

Electronic journal (list all authors)

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

Patent (list all authors)

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

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

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

Units

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

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

Abbreviations

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

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

Editorial: http://www.wjgnet.com/1948-9358/g_info_20100316080002.htm

Frontier: http://www.wjgnet.com/1948-9358/g_info_20100316091946.htm

Topic highlight: http://www.wjgnet.com/1948-9358/g_info_20100316080004.htm

Observation: http://www.wjgnet.com/1948-9358/g_info_20100107142558.htm

Guidelines for basic research: http://www.wjgnet.com/1948-9358/g_info_20100316092358.htm

Guidelines for clinical practice: http://www.wjgnet.com/1948-9358/g_info_20100316092508.htm

Review: http://www.wjgnet.com/1948-9358/g_info_20100107142809.htm

Original articles: http://www.wjgnet.com/1948-9358/g_info_20100107143306.htm

Brief articles: http://www.wjgnet.com/1948-9358/g_info_20100316093137.htm

Case report: http://www.wjgnet.com/1948-9358/g_info_20100107143856.htm

Letters to the editor: http://www.wjgnet.com/1948-9358/g_info_20100107144156.htm

Book reviews: http://www.wjgnet.com/1948-9358/g_info_20100316093525.htm

Guidelines: http://www.wjgnet.com/1948-9358/g_info_20100316093551.htm

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

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

Editorial Office
World Journal of Diabetes
Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjd@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893

Language evaluation

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

Copyright assignment form

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

Responses to reviewers

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

Proof of financial support

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

Links to documents related to the manuscript

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

Science news releases

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

Publication fee

WJD is an international, peer-reviewed, Open-Access, online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. The related standards are as follows. Publication fee: 1300 USD per article. Editorial, topic highlights, original articles, book reviews and letters to the editor are published free of charge.