Open
ORIGINAL ARTICLE

Cell Research (2017) :1-18.
www.nature.com/cr

Intermittent fasting promotes adipose thermogenesis and metabolic homeostasis via VEGF-mediated alternative activation of macrophage

Kyoung-Han Kim[1, 12, *], Yun Hye Kim[2, *], Joe Eun Son[1], Ju Hee Lee[2, 3], Sarah Kim[2], Min Seon Choe[1], Joon Ho Moon[2], Jian Zhong[2], Kiya Fu[2], Florine Lenglin[2], Jeong-Ah Yoo[2], Philip J Bilan[4], Amira Klip[4], Andras Nagy[5, 6], Jae-Ryong Kim[7], Jin Gyoon Park[8], Samer MI Hussein[9], Kyung-Oh Doh[10], Chi-chung Hui[11], Hoon-Ki Sung[2, 3]

[1]Developmental & Stem Cell Biology Program, The Hospital for Sick Children, Toronto, Ontario, Canada; [2]Translational Medicine Program, The Hospital for Sick Children, Toronto, Ontario, Canada; [3]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada; [4]Cell Biology Program, The Hospital for Sick Children, Toronto, Ontario, Canada; [5]Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada; [6]Department of Obstetrics & Gynaecology and Institute of Medical Science, University of Toronto, Toronto, Ontario, Canada; [7]Department of Biochemistry and Molecular Biology, Smart-aging Convergence Research Center, College of Medicine, Yeungnam University, Daegu, Republic of Korea; [8]Virginia G. Piper Center for Personalized Diagnostics, Biodesign Institute, Arizona State University, Tempe, AZ, USA; [9]Centre Hospitalier Universitaire de Québec Research Center and Faculty of Medicine, Laval University, Quebec City, Quebec, Canada; [10]Department of Physiology, College of Medicine, Yeungnam University, Daegu, Republic of Korea; [11]Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada

Intermittent fasting (IF), a periodic energy restriction, has been shown to provide health benefits equivalent to prolonged fasting or caloric restriction. However, our understanding of the underlying mechanisms of IF-mediated metabolic benefits is limited. Here we show that isocaloric IF improves metabolic homeostasis against diet-induced obesity and metabolic dysfunction primarily through adipose thermogenesis in mice. IF-induced metabolic benefits require fasting-mediated increases of vascular endothelial growth factor (VEGF) expression in white adipose tissue (WAT). Furthermore, periodic adipose-VEGF overexpression could recapitulate the metabolic improvement of IF in non-fasted animals. Importantly, fasting and adipose-VEGF induce alternative activation of adipose macrophage, which is critical for thermogenesis. Human adipose gene analysis further revealed a positive correlation of adipose VEGF-M2 macrophage-WAT browning axis. The present study uncovers the molecular mechanism of IF-mediated metabolic benefit and suggests that isocaloric IF can be a preventive and therapeutic approach against obesity and metabolic disorders.

***Keywords*:** intermittent fasting; thermogenesis; vascular endothelial growth factor; adipose macrophage
Cell Research advance online publication 17 October 2017; doi:10.1038/cr.2017.126

Introduction

While fat (white adipose tissue, WAT) is often associated with development of obesity and type 2 diabetes, it is essential for energy homeostasis by storing excess energy and releasing lipids in response to energy deficits [1, 2]. Recent studies have discovered that WAT also contributes to whole-body metabolism by regulating thermogenic activity via the browning of WAT, which increases energy expenditure and improves insulin sensitivity [3]. In this regard, WAT browning has been suggested as a therapeutic approach for obesity and metabolic diseases.

*These two authors contributed equally to this work.
Correspondence: Chi-chung Hui[a], Hoon-Ki Sung[b]
[a]E-mail: cchui@sickkids.ca
[b]E-mail: hoon-ki.sung@sickkids.ca
[12]Current address: University of Ottawa Heart Institute and Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada
Received 3 March 2017; revised 30 June 2017; accepted 26 July 2017

dotoxemia and obesity-associated resistance to insulin. *Nat Immunol* 2014; **15**:423-430.
52 Brandhorst S, Choi IY, Wei M, *et al.* A periodic diet that mimics fasting promotes multi-system regeneration, enhanced cognitive performance, and healthspan. *Cell Metab* 2015; **22**:86-99.
53 Hatori M, Vollmers C, Zarrinpar A, *et al.* Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metab* 2012; **15**:848-860.
54 Cao Y. Angiogenesis and vascular functions in modulation of obesity, adipose metabolism, and insulin sensitivity. *Cell Metab* 2013; **18**:478-489.
55 Shimizu I, Aprahamian T, Kikuchi R, *et al.* Vascular rarefaction mediates whitening of brown fat in obesity. *J Clin Invest* 2014; **124**:2099-2112.
56 Robciuc MR, Kivela R, Williams IM, *et al.* VEGFB/VEGFR1-induced expansion of adipose vasculature counteracts obesity and related metabolic complications. *Cell Metab* 2016; **23**:712-724.
57 Fredriksson JM, Nikami H, Nedergaard J. Cold-induced expression of the VEGF gene in brown adipose tissue is independent of thermogenic oxygen consumption. *FEBS Lett* 2005; **579**:5680-5684.
58 Seki T, Hosaka K, Lim S, *et al.* Endothelial PDGF-CC regulates angiogenesis-dependent thermogenesis in beige fat. *Nat Commun* 2016; **7**:12152.
59 Fabbiano S, Suarez-Zamorano N, Rigo D, *et al.* Caloric restriction leads to browning of white adipose tissue through type 2 immune signaling. *Cell Metab* 2016; **24**:434-446.
60 Man K, Kutyavin VI, Chawla A. Tissue immunometabolism: development, physiology, and pathobiology. *Cell Metab* 2017; **25**:11-26.
61 Molofsky AB, Nussbaum JC, Liang HE, *et al.* Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *J Exp Med* 2013; **210**:535-549.
62 Zhou Y, Yu X, Chen H, *et al.* Leptin deficiency shifts mast cells toward anti-inflammatory actions and protects mice from obesity and diabetes by polarizing M2 macrophages. *Cell Metab* 2015; **22**:1045-1058.
63 Cao R, Brakenhielm E, Wahlestedt C, Thyberg J, Cao Y. Leptin induces vascular permeability and synergistically stimulates angiogenesis with FGF-2 and VEGF. *Proc Natl Acad Sci USA* 2001; **98**:6390-6395.
64 Lo JC, Ljubicic S, Leibiger B, *et al.* Adipsin is an adipokine that improves β cell function in diabetes. *Cell* 2014; **158**:41-53.
65 Heilbronn LK, Smith SR, Martin CK, Anton SD, Ravussin E. Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. *Am J Clin Nutr* 2005; **81**:69-73.
66 Wei M, Brandhorst S, Shelehchi M, *et al.* Fasting-mimicking diet and markers/risk factors for aging, diabetes, cancer, and cardiovascular disease. *Sci Transl Med* 2017; **9**.
67 Ellacott KL, Morton GJ, Woods SC, Tso P, Schwartz MW. Assessment of feeding behavior in laboratory mice. *Cell Metab* 2010; **12**:10-17.
68 Harvie M, Howell A. Potential benefits and harms of intermittent energy restriction and intermittent fasting amongst obese, overweight and normal weight subjects - a narrative review of human and animal evidence. *Behav Sci* 2017; **7**.
69 LeCheminant JD, Christenson E, Bailey BW, Tucker LA. Restricting night-time eating reduces daily energy intake in healthy young men: a short-term cross-over study. *Br J Nutr* 2013; **110**:2108-2113.
70 Thaiss CA, Zeevi D, Levy M, *et al.* Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell* 2014; **159**:514-529.
71 Kong X, Banks A, Liu T, *et al.* IRF4 is a key thermogenic transcriptional partner of PGC-1alpha. *Cell* 2014; **158**:69-83.
72 Muzumdar MD, Tasic B, Miyamichi K, Li L, Luo L. A global double-fluorescent Cre reporter mouse. *Genesis* 2007; **45**:593-605.

(**Supplementary information** is linked to the online version of the paper on the *Cell Research* website.)

SCIENTIFIC REPORTS

OPEN

Thermogenesis-independent metabolic benefits conferred by isocaloric intermittent fasting in *ob/ob* mice

Received: 10 July 2018
Accepted: 23 January 2019
Published online: 21 February 2019

Yun Hye Kim[1], Ju Hee Lee[1,2], Joanna Lan-Hing Yeung[1], Eashita Das[1,3], Ri Youn Kim[4,5], Yanqing Jiang[1], Joon Ho Moon[1], Hyerin Jeong[1], Nikita Thakkar[1], Joe Eun Son[6], Natasha Trzaskalski[4,7], Chi-chung Hui[6,8], Kyung-Oh Doh[9], Erin E. Mulvihill[4,7], Jae-Ryong Kim[10], Kyoung-Han Kim[4,5] & Hoon-Ki Sung[1,2,11]

Intermittent fasting (IF) is an effective dietary intervention to counteract obesity-associated metabolic abnormalities. Previously, we and others have highlighted white adipose tissue (WAT) browning as the main underlying mechanism of IF-mediated metabolic benefits. However, whether IF retains its efficacy in different models, such as genetically obese/diabetic animals, is unknown. Here, leptin-deficient *ob/ob* mice were subjected to 16 weeks of isocaloric IF, and comprehensive metabolic phenotyping was conducted to assess the metabolic effects of IF. Unlike our previous study, isocaloric IF-subjected *ob/ob* animals failed to exhibit reduced body weight gain, lower fat mass, or decreased liver lipid accumulation. Moreover, isocaloric IF did not result in increased thermogenesis nor induce WAT browning in *ob/ob* mice. These findings indicate that isocaloric IF may not be an effective approach for regulating body weight in *ob/ob* animals, posing the possible limitations of IF to treat obesity. However, despite the lack of improvement in insulin sensitivity, isocaloric IF-subjected *ob/ob* animals displayed improved glucose tolerance as well as higher postprandial insulin level, with elevated incretin expression, suggesting that isocaloric IF is effective in improving nutrient-stimulated insulin secretion. Together, this study uncovers the insulinotropic effect of isocaloric IF, independent of adipose thermogenesis, which is potentially complementary for the treatment of type 2 diabetes.

Over the past few decades, the prevalence of obesity has dramatically increased across all genders and age groups, reaching a global epidemic level. As obesity is strongly associated with the development of other chronic health conditions, such as type 2 diabetes, hypertension, and non-alcoholic fatty liver disease (NAFLD), development of feasible and practical treatments to counteract obesity is urgently needed. A number of factors contribute to obesity, including genetic determinants, environmental and behavioural traits[1–3]. In particular, polymorphisms in various genes regulating appetite and metabolic rate were identified to predispose individuals to obesity.

[1]Translational Medicine Program, The Hospital for Sick Children, Toronto, Ontario, Canada. [2]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada. [3]Department of Microbiology, Siliguri College, North Bengal University, West Bengal, India. [4]University of Ottawa Heart Institute, Ottawa, Ontario, Canada. [5]Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario, Canada. [6]Developmental and Stem Cell Biology Program, The Hospital for Sick Children, Toronto, Ontario, Canada. [7]Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Ontario, Canada. [8]Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada. [9]Department of Physiology, College of Medicine, Yeungnam University, Gyeongsan, Republic of Korea. [10]Department of Biochemistry and Molecular Biology & Smart-aging Convergence Research Center, College of Medicine, Yeungnam University, Gyeongsan, Republic of Korea. [11]Banting and Best Diabetes Centre, University of Toronto, Toronto, Ontario, Canada. Yun Hye Kim and Ju Hee Lee contributed equally. Correspondence and requests for materials should be addressed to J.-R.K. (email: kimjr000@gmail.com) or K.-H.K. (email: hankim@uottawa.ca) or H.-K.S. (email: hoon-ki.sung@sickkids.ca)

32. Dubuc, P. U., Wilden, N. J. & Carlisle, H. J. Fed and fasting thermoregulation in ob/ob mice. *Ann Nutr Metab* **29**, 358–365, https://doi.org/10.1159/000176992 (1985).
33. Dodd, G. T. *et al.* Leptin and insulin act on POMC neurons to promote the browning of white fat. *Cell* **160**, 88–104, https://doi.org/10.1016/j.cell.2014.12.022 (2015).
34. Lanier, V. *et al.* Leptin-induced transphosphorylation of vascular endothelial growth factor receptor increases Notch and stimulates endothelial cell angiogenic transformation. *Int J Biochem Cell Biol* **79**, 139–150, https://doi.org/10.1016/j.biocel.2016.08.023 (2016).
35. Park, H. Y. *et al.* Potential role of leptin in angiogenesis: leptin induces endothelial cell proliferation and expression of matrix metalloproteinases *in vivo* and *in vitro*. *Exp Mol Med* **33**, 95–102, https://doi.org/10.1038/emm.2001.17 (2001).
36. Yang, W. H. *et al.* Leptin increases VEGF expression and enhances angiogenesis in human chondrosarcoma cells. *Biochim Biophys Acta* **1840**, 3483–3493, https://doi.org/10.1016/j.bbagen.2014.09.012 (2014).
37. Cao, R., Brakenhielm, E., Wahlestedt, C., Thyberg, J. & Cao, Y. Leptin induces vascular permeability and synergistically stimulates angiogenesis with FGF-2 and VEGF. *Proc Natl Acad Sci USA* **98**, 6390–6395, https://doi.org/10.1073/pnas.101564798 (2001).
38. Kuo, L. E. *et al.* Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. *Nat Med* **13**, 803–811, https://doi.org/10.1038/nm1611 (2007).
39. Ley, R. E. *et al.* Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* **102**, 11070–11075, https://doi.org/10.1073/pnas.0504978102 (2005).
40. Secor, S. M. & Carey, H. V. Integrative Physiology of Fasting. *Compr Physiol* **6**, 773–825, https://doi.org/10.1002/cphy.c150013 (2016).
41. Thaiss, C. A. *et al.* Transkingdom control of microbiota diurnal oscillations promotes metabolic homeostasis. *Cell* **159**, 514–529, https://doi.org/10.1016/j.cell.2014.09.048 (2014).
42. Leibowitz, G., Kaiser, N. & Cerasi, E. beta-Cell failure in type 2 diabetes. *J Diabetes Investig* **2**, 82–91, https://doi.org/10.1111/j.2040-1124.2010.00094.x (2011).
43. Liu, H. *et al.* Intermittent fasting preserves beta-cell mass in obesity-induced diabetes via the autophagy-lysosome pathway. *Autophagy* **13**, 1952–1968, https://doi.org/10.1080/15548627.2017.1368596 (2017).
44. Yoneshiro, T. *et al.* Age-related decrease in cold-activated brown adipose tissue and accumulation of body fat in healthy humans. *Obesity (Silver Spring)* **19**, 1755–1760, https://doi.org/10.1038/oby.2011.125 (2011).
45. Seale, P. & Lazar, M. A. Brown fat in humans: turning up the heat on obesity. *Diabetes* **58**, 1482–1484, https://doi.org/10.2337/db09-0622 (2009).
46. Stote, K. S. *et al.* A controlled trial of reduced meal frequency without caloric restriction in healthy, normal-weight, middle-aged adults. *Am J Clin Nutr* **85**, 981–988, https://doi.org/10.1093/ajcn/85.4.981 (2007).
47. Folch, J., Lees, M. & Sloane Stanley, G. H. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* **226**, 497–509 (1957).

Acknowledgements

For this study, H.K.S. was supported by grants from Pilot and Feasibility Study Grant of Banting & Best Diabetes Centre (BBDC) and The Hospital for Sick Children Start-up fund; K.H.K was supported by the Heart and Stroke Foundation of Canada Grant-in-Aid (G-18-0022213) and the University of Ottawa Heart Institute Start-up fund; E.E.M. is a Diabetes Canada New Investigator and was supported by a grant from the Canadian Institutes of Health Research (PJT156136); C.C.H. was supported by a grant from the Canadian Institutes of Health Research; J.R.K. was supported by Medical Research Center Program (2015R1A5A2009124) through National Research Foundation of Korea (NRF) funded by Ministry of Science and ICT. K.O.D. was supported by the Creative Economy Leading Technology Development Program through the Gyeongsangbuk-Do and Gyeongbuk Science & Technology Promotion Center of Korea (SF316001A). J.E.S. is supported by a fellowship from the Diabetes Canada. J.H.L. is supported by the Restracomp scholarship from The Hospital for Sick Children. R.Y.K. is supported by a fellowship from the University of Ottawa Cardiology Research Endowment Fund.

Author Contributions

K.H.K., H.K.S. and J.R.K. designed the project. J.H.L., K.H.K., H.K.S. and Y.H.K. wrote the manuscript. Y.H.K., J.H.L., J.E.S., E.D., Y.J. and J.H.M. performed mouse metabolic experiments. Y.H.K. analysed mouse metabolic data. J.H.L., Y.H.K. and J.L.Y. performed gene expression analysis, with assistance from Y.J. Tissue process and histology staining were conducted and analyzed by J.L.Y., Y.J., H.J. and N.T. R.Y.K. and N.T. performed plasma incretin level analysis. C.C.H., K.O.D. and E.M. provided scientific discussion and technical support. All authors contributed to the discussion and commented on the manuscript.

Additional Information

Supplementary information accompanies this paper at https://doi.org/10.1038/s41598-019-39380-2.

Competing Interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.