[16] M. Matsuda, R.A. DeFronzo, Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp, Diabetes Care 22 (1999) 1462–1470.
[17] Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National Diabetes Data Group, Diabetes 28 (1979) 1039-1057.
[18] A. Taniguchi, M. Fukushima, M. Sakai, K. Miwa, T. Makita, I. Nagata, et al. Remnant-like particle cholesterol, triglycerides, and insulin resistance in nonobese Japanese type 2 diabetic patients, Diabetes Care 23 (2000) 1766–1769.
[19] D. Tripathy, M. Carlsson, P. Almgren, B. Isomaa, M.R. Taskinen, T. Tuomi, et al. Insulin secretion and insulin sensitivity in relation to glucose tolerance: lessons from the Botnia Study, Diabetes 49 (2000) 975–980.
[20] A. Kuroe, M. Fukushima, M. Usami, M. Ikeda, Y. Nakai, A. Taniguchi, et al. Impaired beta-cell function and insulin sensitivity in Japanese subjects with normal glucose tolerance, Diabetes Res. Clin. Pract. 59 (2003) 71–77.
[21] H. Suzuki, M. Fukushima, M. Usami, M. Ikeda, A. Taniguchi, Y. Nakai, et al. Factors responsible for development from normal glucose tolerance to isolated postchallenge hyperglycemia, Diabetes Care 26 (2003) 1211–1215.
[22] M. Hanefeld, C. Koehler, K. Fuecker, E. Henkel, F. Schaper, T. Temelkova-Kurktschiev, Insulin secretion and insulin sensitivity pattern is different in isolated impaired glucose tolerance and impaired fasting glucose: the risk factor in impaired glucose tolerance for atherosclerosis and diabetes study, Diabetes Care 26 (2003) 868–874.
[23] T. Ohmura, K. Ueda, Y. Kiyohara, I. Kato, H. Iwamoto, K. Nakayama, et al. The association of the insulin resistance syndrome with impaired glucose tolerance and NIDDM in the Japanese general population: the Hisayama study, Diabetologia 37 (1994) 897–904.
[24] M. Fukushima, M. Usami, M. Ikeda, Y. Nakai, A. Taniguchi, T. Matsuura, et al. Insulin Secretion and Insulin Sensitivity at Different Stages of Glucose Tolerance: a cross-sectional study of Japanese type 2 diabetes, Metabolism 53 (2004) 831–835.
[25] A. Sekikawa, M. Tominaga, K. Takahashi, H. Eguchi, M. Igarashi, H. Ohnuma, et al. Prevalence of diabetes and impaired glucose tolerance in funagata area, Japan, Diabetes Care 16 (1993) 570–574.
[26] Q. Qiao, T. Nakagami, J. Tuomilehto, K. Borch-Johnsen, B. Balkau, Y. Iwamoto, et al. The DECODA Study Group on behalf of the International Diabetes Epidemiology Group, Comparison of the fasting and 2-h glucose criteria for diabetes in different Asian cohorts, Diabetologia 43 (2000) 1470–1475.
[27] M. Fukushima, H. Suzuki, Y. Seino, Insulin secretion capacity in the development from normal glucose tolerance to type 2 diabetes, Diabetes Res. Clin. Pract. 66 (Suppl. 1) (2004) S37–S43.
[28] A. Mandavilli, D. Cyranoski, Asia's big problem, Nat. Med. 10 (2004) 325–327.
[29] C.C. Jensen, M. Cnop, R.L. Hull, W.Y. Fujimoto, SE. Kahn, American Diabetes Association GENNID Study Group, β-cell function is a major contributor to oral glucose tolerance in high-risk relatives of four ethnic groups in the U.S., Diabetes 51 (2002) 2170–2178.
[30] G.J. Blake, P.M. Ridker, Novel clinical markers of vascular wall inflammation, Circ. Res. 89 (2001) 763–771.
[31] A. Festa, R. D'Agostino Jr., G. Howard, L. Mykkanen, R.P. Tracy, S.M. Haffner, Chronic subclinical inflammation as part of the insulin resistance syndrome, the Insulin Resistance Atherosclerosis Study (IRAS), Circulation 102 (2000) 42–47.
[32] J.S. Yudkin, C.D. Stehouwer, J.J. Emeis, S.W. Coppack, C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler. Thromb. Vasc. Biol. 19 (1999) 972–978.
[33] B. Vozarova, C. Weyer, K. Hanson, P.A. Tataranni, C. Bogardus, R.E. Pratley, Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion, Obes. Res. 9 (2001) 414–417.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 335 (2005) 937–942
BBRC
www.elsevier.com/locate/ybbrc

Gastric inhibitory polypeptide modulates adiposity and fat oxidation under diminished insulin action

Heying Zhou [a], Yuichiro Yamada [a,*], Katsushi Tsukiyama [a], Kazumasa Miyawaki [a], Masaya Hosokawa [a], Kazuaki Nagashima [a], Kentaro Toyoda [a], Rei Naitoh [a], Wataru Mizunoya [b], Tohru Fushiki [b], Takashi Kadowaki [c], Yutaka Seino [a,d]

[a] *Department of Diabetes and Clinical Nutrition, Kyoto University Graduate School of Medicine, Kyoto, Japan*
[b] *Division of Food Sciences and Biotechnology, Kyoto University Graduate School of Agriculture, Kyoto, Japan*
[c] *Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo, Tokyo, Japan*
[d] *Kansai-Denryoku Hospital, Osaka, Japan*

Received 27 July 2005
Available online 9 August 2005

Abstract

Gut hormone gastric inhibitory polypeptide (GIP) stimulates insulin secretion from pancreatic β-cells upon ingestion of nutrients. Inhibition of GIP signaling prevents the onset of obesity and consequent insulin resistance induced by high-fat diet. In this study, we investigated the role of GIP in accumulation of triglycerides into adipocytes and in fat oxidation peripherally using insulin receptor substrate (IRS)-1-deficient mice and revealed that *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice exhibited both reduced adiposity and ameliorated insulin resistance. Furthermore, increased gene expression of CD36 and UCP2 in liver, and increased expression and enzyme activity of 3-hydroxyacyl-CoA dehydrogenase in skeletal muscle of *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice might contribute to the lower respiratory quotient and the higher fat oxidation in light phase. These results suggest that GIP plays a crucial role in switching from fat oxidation to fat accumulation under the diminished insulin action as a potential target for secondary prevention of insulin resistance.

Keywords: GIP; IRS-1; PPARα; Energy expenditure

Glucose homeostasis is tightly controlled in vivo by the levels of insulin secretion and peripheral insulin sensitivity. Insulin is released from pancreatic β-cells in proportion to increasing concentrations of glucose, lowering blood glucose levels by stimulating glucose uptake into skeletal muscle and adipose tissue and by decreasing glucose production by the liver. Type 2 diabetes is characterized by both decreased insulin sensitivity and impaired insulin secretion, which results in persistent hyperglycemia of sufficient magnitude to produce diabetic complications such as retinopathy, nephropathy, and neuropathy [1,2].

Glucose is metabolized in pancreatic β-cells, resulting an increase in the ATP/ADP ratio, closing the ATP-sensitive potassium (K_{ATP}) channels, causing plasma membrane depolarization, influx of Ca^{2+}, and insulin exocytosis [3,4]. In addition to this direct effect of glucose, hormonal factor(s) called incretins transmit signals from the gut to the pancreatic β-cells and play an important role in glucose homeostasis by promoting insulin secretion immediately upon meal ingestion [5,6].

Gastric inhibitory polypeptide (GIP), an incretin also called glucose-dependent insulinotropic polypeptide, is released from duodenal endocrine K cells to stimulate

* Corresponding author. Fax: +81 75 751 3677.
E-mail address: yamada@metab.kuhp.kyoto-u.ac.jp (Y. Yamada).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.07.164

insulin secretion from pancreatic β-cells upon absorption of nutrients [7]. Homozygous mice lacking GIP receptor ($GIPR^{-/-}$) exhibit impaired initial insulin secretion and hyperglycemia after oral glucose loading [8].

The GIP receptor is expressed in various tissues including the pancreatic islets, adipose tissue, and brain [9]. In adipocytes, GIP stimulates cellular uptake of 2-deoxy-D-glucose and increases heparin-releasable lipoprotein lipase activity in the presence of insulin [10,11]. However, $GIPR^{-/-}$ mice have similar body weight [8] and the physiological role of GIP is, therefore, little in fat uptake into adipocytes under the normal diet.

Insulin signaling is responsible for fat uptake both into adipocytes where fat is stored, and into liver and skeletal muscle where fat is utilized as energy. We have previously shown that high-fat feeding in mice with normal insulin sensitivity results in extreme visceral and subcutaneous fat deposition and insulin resistance, and inhibition of GIP signaling protects from such obesity and insulin resistance [11]. As plasma GIP levels are exaggerated by high-fat diet, increased GIP signaling promotes fat accumulation into adipocytes rather than fat oxidation in such a situation. Therefore, GIP signaling is a potential target for primary prevention of obesity and consequent insulin resistance induced by high-fat diet.

In the present study, we investigated the role of GIP in the accumulation of triglycerides into adipocytes under diminished insulin action using insulin receptor substrate (IRS)-1-deficient knockout mice [12] and revealed that GIP plays a crucial role in switching from fat oxidation to fat accumulation in the state with the diminished insulin action and that GIP signaling is a potential target for secondary prevention of insulin resistance.

Materials and methods

Animals. The generation of $GIPR^{-/-}$ and $IRS\text{-}1^{-/-}$ mice (C57BL/6 background) has been described previously [8,12]. Heterozygous mutants for the IRS-1 gene ($IRS\text{-}1^{+/-}$) were crossed with heterozygous mutants for the GIPR gene ($GIPR^{+/-}$), and then $IRS\text{-}1^{+/-}$ $GIPR^{+/-}$ mice were further bred. Genotyping was done by PCR. Only male $IRS\text{-}1^{-/-}$ $GIPR^{+/+}$ and $IRS\text{-}1^{-/-}$ $GIPR^{-/-}$ mice were used in the experiments. Mice were bred on normal chow and maintained using standard procedures. Animal care and procedures were approved by the Animal Care Committee of Kyoto University Graduate School of Medicine.

Glucose tolerance test. Male mice (10–20 weeks of age) were fasted for more than 16 h before the study. Glucose (2 g/kg body weight) was then loaded orally. Blood samples were taken at the indicated times. Blood glucose levels were measured by the enzyme-electrode method. Plasma insulin levels were measured using an ELISA kit (Shibayagi, Gunma, Japan).

Insulin tolerance test. Male mice (10–20 weeks of age) were fed ad lib and intraperitoneally challenged with insulin (0.3 U/kg body weight). Blood samples were taken at the indicated times and measured as described above.

Measurement of leptin and free fatty acid levels. Blood samples were collected in heparinized capillary tubes from the tail vein and centrifuged at 3000 rpm. Plasma leptin levels were measured using an ELISA kit (Morinaga, Yokohama, Japan). Plasma free fatty acid levels were determined using a NEFA kit (Wako, Osaka, Japan).

Energy expenditure. Oxygen consumption and respiratory quotient were measured by indirect calorimetric system every 13 min for 24 h in mice under fed condition, as described previously [11,13]. Briefly, air from the room was pumped through the chamber, and the expired gas was filtered through thin cotton, which was dried and subjected to a gas analyzer (Alco System model RL-600, Chiba, Japan). The O_2 and CO_2 concentrations were measured and the respiratory quotient was calculated using software for analysis (Alco System). Fat oxidation was calculated in a formula of $1.67 \times (1 - \text{respiratory quotient}) \times \text{oxygen consumption}$ as described previously [13].

Quantitative RT-PCR. Total RNA was isolated from liver and muscle with Trizol reagent (Invitrogen), and gene expression levels were corrected for GAPDH mRNA level and were examined by real time quantitative RT-PCR using a PE Applied Biosystems prism model 7000 sequence detection instrument. Primers and a TaqMan probe for each gene were designed using PRIMER EXPRESS software package (Applied Biosystems, CA) from gene sequences obtained from GenBank (Table 1).

Activity of 3-hydroxyacyl-CoA dehydrogenase. The muscle was quickly removed, weighed, and immediately homogenized in 20 volumes of ice-cold 5.4 M glycerol, 5 mM 2-mercaptoethanol, 0.5 mM EDTA, 0.02% bovine serum albumin, and 20 mM phosphate buffer (pH 7.4). The homogenate was centrifuged at 600*g* for 10 min, and the supernatant was used for enzyme assays. All homogenate operations were performed at 0–4 °C. The assay system of 3-hydroxyacyl-CoA dehydrogenase (HD) activity contained the following: 95 mM pyrophosphate buffer (pH 7.3), 0.2 mM NADH, 4.7 mM acetoacetyl-CoA, and supernatant of homogenate. The supernatant was added, and the rate of decrease in extinction at 339 nm was measured for 2 min at 25 °C [14].

Statistical analyses. Data are expressed as means ± SE. Statistical analyses were performed by unpaired *t* test.

Results

Development of $IRS\text{-}1^{-/-}$ $GIPR^{-/-}$ mice

$IRS\text{-}1^{-/-}$ $GIPR^{+/+}$ and $IRS\text{-}1^{-/-}$ $GIPR^{-/-}$ mice were generated by intercrossing $IRS\text{-}1^{+/-}$ $GIPR^{+/-}$ mice. No significant difference in body weight was observed

Table 1
Primers and probes for real time quantitative RT-PCR

Target	Forward primer	Reverse primer	Probe
CD36	GAGCCCACAGTTCCGATCA	CTGGGAGTTGGCGAGAAAAC	CTCCTCGTGCAGCAGAATCAAGGGA
UCP2	TCCCTTGCCACTTCACTTCTG	TCTCGTCTTGACCACATCA ACAG	TTCTGCACCACCGTCATCGCCTC
ACO/AOX	TCACAGCAGTGGGATTCCAA	TCTGCAGCATCATAACAGTGTTCTC	CACGTTTACCCCGGCCTGCACC
HD	AGACAGGTAAGGGCTGGTATCAGT	AACTCAGAAAGCCAGGGATCAG	TGACAAGCCACTGGGTCGCATCC
CPT-1	TGGCAAAGGTCTTATCAAGAAGTG	CCTCTCGGAACATTCTTGTCATC	CAGAACTTGCCTTTGTCCCGGAAATG
PPARα	CCCTGAACATCGAGTGTCGAA	TTGCAGCTCCGATCACACTT	TCGCCGAAAGAAGCCCTTACAGCCTT
PPARγ	TGTCGGTTTCAGAAGTGCCTT	GCTCGCAGATCAGCAGACTCT	CCCAAACCTGATGGCATTGTGAGACA

between *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ and *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice fed a normal diet, although both mice exhibited about 34% less body weight than wild-type (*IRS-1*$^{+/+}$ *GIPR*$^{+/+}$) mice (data not shown), consistent with the previous finding [12].

The fasting blood glucose did not show a significant difference between *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ and *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice. After oral glucose loading, *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice had similar blood glucose levels compared to *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ mice, except for the early phase (15 min after glucose loading) (Fig. 1A). *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice exhibited significantly decreased plasma insulin levels in the early phase of glucose loading (Fig. 1B). These results indicate that GIP also functions as an insulinotropic signal under diminished insulin action.

Disruption of GIP signaling ameliorates insulin resistance

Intraperitoneal insulin tolerance test was performed to determine the influence of GIP signaling on insulin resistance. Expectedly, *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ mice showed marked insulin resistance, compared with *IRS-1*$^{+/+}$ *GIPR*$^{+/+}$ mice. The drop in blood glucose levels in *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice was more obvious (Fig. 1C), showing that disruption of GIP signaling partially ameliorates the insulin resistance in IRS-1 knockout mice. These results indicate that GIP has the antithetical effects in glucose tolerance.

Fat used as the preferred energy substrate

We reported previously that interruption of the GIP signaling pathway prevents high-fat diet-induced obesity [11]. Dissection of the fat mass of *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice revealed that disruption of GIP signaling elicited a significant reduction in both visceral and subcutaneous fat mass even under the normal diet (Fig. 2A). Consistently, plasma leptin levels, which correlate well with body fat mass, were significantly lower in *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice than in *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ mice (2.23 ± 0.29 ng/ml vs 6.64 ± 1.49 ng/ml, $P < 0.05$).

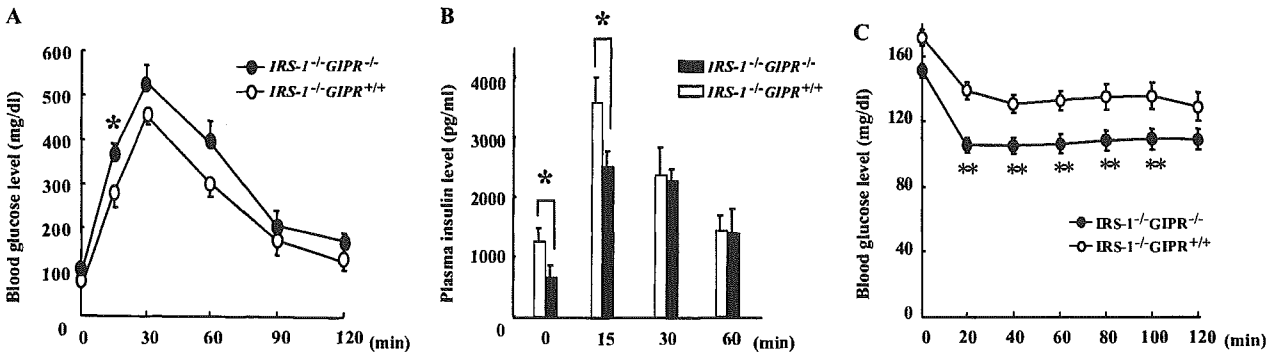

Fig. 1. Glucose tolerance test and insulin tolerance test. (A) Oral glucose tolerance test in age-matched *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ (open circle) and *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice (filled circle). (B) Plasma insulin levels after oral glucose loading for age-matched *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ (open bars) and *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice (filled bars). (C) Blood glucose levels during insulin tolerance test in *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ (open circle) and *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ (filled circle) mice. Values are means ± SE. $*P < 0.05$ for *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice vs *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ mice. $**P < 0.01$.

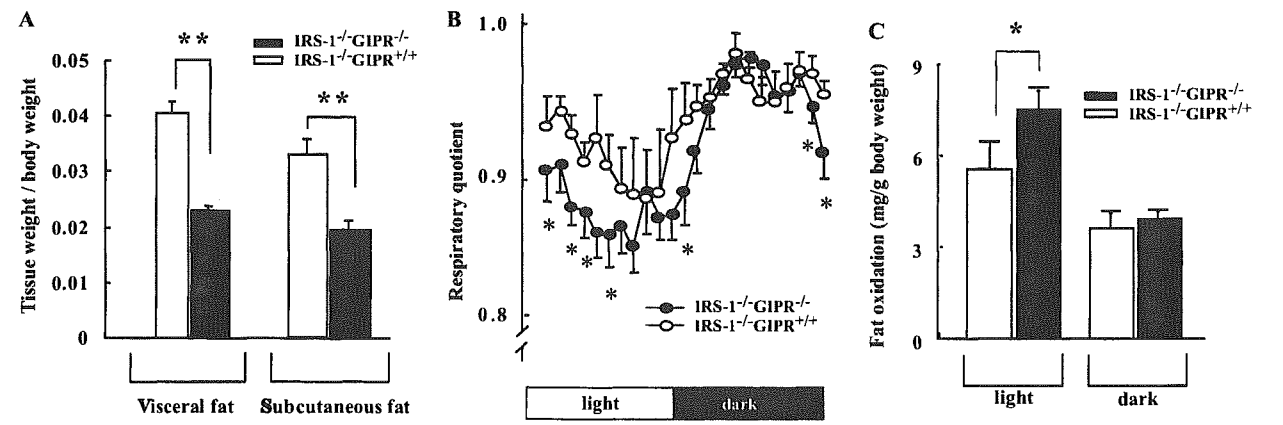

Fig. 2. Adiposity and energy expenditure. (A) Visceral and subcutaneous adipose tissues from *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ (open bar) and *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ (filled bar) mice fed normal diet. Respiratory quotient (B) and calculated fat oxidation (C) under normal diet in *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ (open) and *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ (filled). $*P < 0.05$. $**P < 0.01$.

We then evaluated energy expenditure by measuring respiratory quotient and oxygen consumption by indirect calorimetry. Under the normal diet, respiratory quotients (Fig. 2B) of *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ and *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice were 0.93 ± 0.02 and 0.87 ± 0.02 in the light phase and 0.98 ± 0.02 and 0.98 ± 0.01 in the dark phase, respectively, and the calculated fat oxidation was increased in the light phase by disruption of the GIP signal (Fig. 2C).

Increased β-oxidation in liver

We examined the expression levels of genes for fatty acid transport and β-oxidation in liver and found that the expression of CD36 and UCP2 was significantly increased in the liver of *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice compared to *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ mice (Figs. 3A and B). Gene expression of ACO/AOX, CPT-1, PPARα, and PPARγ in liver did not differ significantly. We also found that the addition of fatty acids, which are natural ligands of PPARα [15–18], increased the expression level of UCP2 using primary isolated hepatocytes (data not shown), consistent with the regulation of CD36 and UCP2 by PPARα [19–21]. Although plasma free fatty acid levels were not significantly different between *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ and *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice (0.88 ± 0.10 mEq/L vs 0.77 ± 0.08 mEq/L), an increase in the flow of fatty acids into hepatocytes may stimulate β-oxidation in liver through activation of PPARα.

Increased β-oxidation in skeletal muscle

We also examined the expression levels of genes for fatty acid transport and β-oxidation in skeletal muscle, and found that expression of HD was significantly increased in skeletal muscle of *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ compared to *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ mice (Fig. 3C). In contrast to the liver, expression of CD36 and UCP2 was not increased in skeletal muscle (data not shown).

Enzyme activities for skeletal muscle HD were also examined (Fig. 3D). HD activity in *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice (0.120 ± 0.005 U/mg) was significantly higher than in *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ mice (0.101 ± 0.006 U/mg).

Discussion

Impaired insulin secretion and insulin resistance are the major etiologic factors of type 2 diabetes. GIP receptor knockout mice showed mild glucose intolerance associated with impaired insulin secretion [8] and IRS-1 knockout mice showed normal glucose tolerance associated with insulin resistance [12,22]. However, double knockout mice had increased blood glucose levels only in the early phase of oral glucose loading, indicating that GIP signaling plays another role in glucose tolerance.

Glucose and fat metabolism are closely related. In addition to its insulinotropic effect, GIP plays an impor-

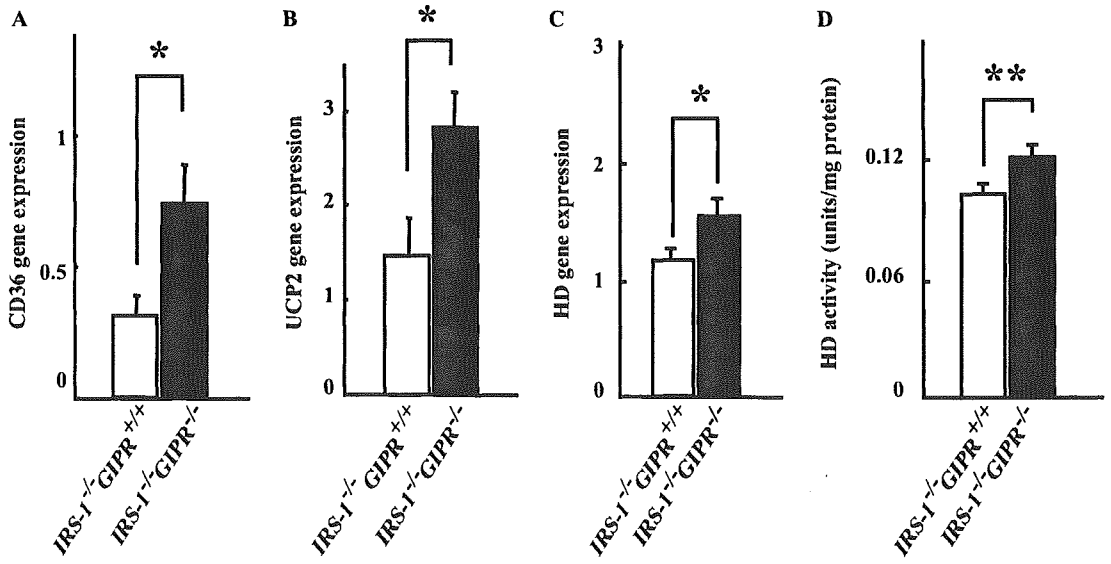

Fig. 3. Gene expression and enzyme activity Gene expression of CD36 (A) and UCP2 (B) in liver. *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ (open) and *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ (filled). Gene expression (C) and enzyme activity (D) of HD in skeletal muscle of *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ (open) and *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ (filled). $^{*}P < 0.05$, $^{**}P < 0.01$.

tant role in nutrient uptake into adipocytes. However, GIP signaling is little involved in fat accumulation into adipocytes under the normal insulin sensitivity, as *GIPR*$^{-/-}$ mice show similar adiposity to wild-type on control diet (Fig. 4A). In contrast, GIP signaling is required for effective accumulation of nutrients under high-fat diet, and inhibition of GIP signaling not only prevents obesity but also insulin resistance. Therefore, inhibition of GIP signaling primarily prevents the onset of obesity and consequent insulin resistance [11].

In this study, we have investigated the role of GIP signaling in triglyceride accumulation into adipocytes under normal dietary conditions under the diminished insulin action (Figs. 4B and C). Insulin receptor substrate (IRS) proteins are the major substrates of insulin receptor tyrosine kinase and mediate pleiotropic effects of insulin including regulation of cell metabolism, survival, growth, and differentiation [23]. Disruption of the IRS-1 gene results in insulin resistance [12,22]. It has been reported that insulin-induced phosphatidylinositol-3-kinase activity in the adipocytes of *IRS-1*$^{-/-}$ mice is about half that of wild-type mice [24]. *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice show significantly reduced adiposity compared to *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ mice, indicating that GIP plays an important role in fat accumulation into adipocytes under the diminished insulin action.

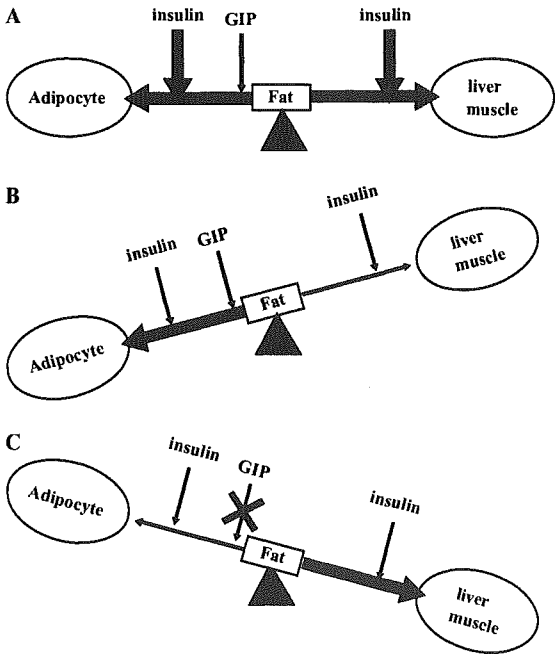

Fig. 4. Schematic models for GIP signaling, adiposity, and fat oxidation. (A) Insulin signaling is responsible for fat accumulation in adipocytes and fat oxidation in liver and skeletal muscle. The effect of GIP signaling is trivial. (B) Under the diminished insulin action, the effect of GIP signaling becomes relatively increased and fat moves toward accumulation in adipocytes. (C) Under the diminished insulin action and inhibition of GIP signaling, fat moves toward oxidation in liver and skeletal muscle.

Inhibition of fat oxidation is correlated with increased intracellular triglyceride content and decreased insulin action [25]. The *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice exhibited a lower respiratory quotient and a higher fat oxidation than *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ mice, especially in the light phase, with similar free fatty acid levels in serum, indicating that fat is not accumulating in adipocytes or in blood, but is being utilized as the preferred energy substrate (Figs. 4B and C). Liver and skeletal muscle where GIP receptors are not expressed are the major sites of whole body fat oxidation. CD36, also known as fatty acid translocase (FAT), is a multispecific integral membrane glycoprotein that has been identified as a facilitator of fatty acid uptake. UCP2 dissipates the proton electrochemical gradient by uncoupling fuel oxidation from ATP production. CD36 and UCP-2, both of which are transcriptionally regulated by PPARα in liver, were increased in *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice compared to *IRS-1*$^{-/-}$ *GIPR*$^{+/+}$ mice. These results suggest that fatty acids activate PPARα transcriptional activity and stimulate fat oxidation in the liver. In contrast to the liver, gene expression and enzyme activity of HD, the rate limiting enzyme of fat oxidation in peroxisomes, were increased in skeletal muscle. The molecular mechanism of such induction is unclear at present.

Overweight individuals frequently develop hypertension, dyslipidemia, or hyperglycemia. Clustering of these symptoms with insulin resistance constitutes metabolic syndrome [26]. Accordingly, improvement of obesity is crucial in treatment to prevent the vascular complications. Reduced adiposity in *IRS-1*$^{-/-}$ *GIPR*$^{-/-}$ mice should contribute to ameliorated insulin resistance and its related diseases associated with similar glucose intolerance and decreased postprandial insulin secretion.

The present study suggests that GIP plays a crucial role in switching from fat oxidation to fat accumulation under the diminished insulin action and inhibition of GIP signaling ameliorated insulin resistance. GIP signaling is a potential target for secondary prevention of insulin resistance.

Acknowledgments

This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and by Health Sciences Research Grants for Comprehensive Research on Aging and Health from the Ministry of Health, Labor and Welfare, Japan.

References

[1] R.A. DeFronzo, The triumvirate: β-cell, muscle, liver. A collusion responsible for NIDDM, Diabetes 37 (1988) 667–687.

[2] D. Porte Jr., Banting lecture 1990. β-cells in type II diabetes mellitus, Diabetes 40 (1991) 166–180.
[3] C.B. Wollheim, G.W. Sharp, Regulation of insulin release by calcium, Physiol. Rev. 61 (1981) 914–973.
[4] F.M. Ashcroft, P. Rorsman, Electrophysiology of the pancreatic β-cell, Prog. Biophys. Mol. Biol. 54 (1989) 87–143.
[5] R.H. Unger, A.M. Eisentraut, Entero-insular axis, Arch. Intern. Med. 123 (1969) 261–266.
[6] W. Creutzfeldt, The incretin concept today, Diabetologia 16 (1979) 75–85.
[7] J.J. Meier, M.A. Nauck, W.E. Schmidt, B. Gallwitz, Gastric inhibitory polypeptide: the neglected incretin revisited, Regul. Pept. 107 (2002) 1–13.
[8] K. Miyawaki, Y. Yamada, H. Yano, H. Niwa, N. Ban, Y. Ihara, A. Kubota, S. Fujimoto, M. Kajikawa, A. Kuroe, K. Tsuda, H. Hashimoto, T. Yamashita, T. Jomori, F. Tashiro, J. Miyazaki, Y. Seino, Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice, Proc. Natl. Acad. Sci. USA 96 (1999) 14843–14847.
[9] T.B. Usdin, E. Mezey, D.C. Button, M.J. Brownstein, T.I. Bonner, Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain, Endocrinology 133 (1993) 2861–2870.
[10] R.G. Yip, M.O. Boylan, T.J. Kieffer, M.M. Wolfe, Functional GIP receptors are present on adipocytes, Endocrinology 139 (1998) 4004–4007.
[11] K. Miyawaki, Y. Yamada, N. Ban, Y. Ihara, K. Tsukiyama, H. Zhou, S. Fujimoto, A. Oku, K. Tsuda, S. Toyokuni, H. Hiai, W. Mizunoya, T. Fushiki, J.J. Holst, M. Makino, A. Tashita, Y. Kobara, Y. Tsubamoto, T. Jinnouchi, T. Jomori, Y. Seino, Inhibition of gastric inhibitory polypeptide signaling prevents obesity, Nat. Med. 8 (2002) 738–742.
[12] H. Tamemoto, T. Kadowaki, K. Tobe, T. Yagi, H. Sakura, T. Hayakawa, Y. Terauchi, K. Ueki, Y. Kaburagi, S. Satoh, et al., Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1, Nature 372 (1994) 182–186.
[13] K. Ishihara, S. Oyaizu, K. Onuki, K. Lim, T. Fushiki, Chronic (−)-hydroxycitrate administration spares carbohydrate utilization and promotes lipid oxidation during exercise in mice, J. Nutr. 130 (2000) 2990–2995.
[14] H.U. Bergmeyer, Methods of Enzymatic Analysis, VCH Publishers, Weinheim, 1983.
[15] S.A. Kliewer, S.S. Sundseth, S.A. Jones, P.J. Brown, G.B. Wisely, C.S. Koble, P. Devchand, W. Wahli, T.M. Willson, J.M. Lenhard, J.M. Lehmann, Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ, Proc. Natl. Acad. Sci. USA 94 (1997) 4318–4323.
[16] G. Krey, O. Braissant, F. L'Horset, E. Kalkhoven, M. Perroud, M.G. Parker, W. Wahli, Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay, Mol. Endocrinol. 11 (1997) 779–791.
[17] B.M. Forman, J. Chen, R.M. Evans, Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ, Proc. Natl. Acad. Sci. USA 94 (1997) 4312–4317.
[18] K. Motojima, P. Passilly, J.M. Peters, F.J. Gonzalez, N. Latruffe, Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor α and γ activators in a tissue- and inducer-specific manner, J. Biol. Chem. 273 (1998) 16710–16714.
[19] L.J. Kelly, P.P. Vicario, G.M. Thompson, M.R. Candelore, T.W. Doebber, J. Ventre, M.S. Wu, R. Meurer, M.J. Forrest, M.W. Conner, M.A. Cascieri, D.E. Moller, Peroxisome proliferator-activated receptors γ and α mediate in vivo regulation of uncoupling protein (UCP-1, UCP-2, UCP-3) gene expression, Endocrinology 139 (1998) 4920–4927.
[20] M.B. Armstrong, H.C. Towle, Polyunsaturated fatty acids stimulate hepatic UCP-2 expression via a PPARα-mediated pathway, Am. J. Physiol. Endocrinol. Metab. 281 (2001) E1197–E1204.
[21] K. Yu, W. Bayona, C.B. Kallen, H.P. Harding, C.P. Ravera, G. McMahon, M. Brown, M.A. Lazar, Differential activation of peroxisome proliferator-activated receptors by eicosanoids, J. Biol. Chem. 270 (1995) 23975–23983.
[22] E. Araki, M.A. Lipes, M.E. Patti, J.C. Bruning, B. Haag III, R.S. Johnson, C.R. Kahn, Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene, Nature 372 (1994) 186–190.
[23] M.F. White, IRS proteins and the common path to diabetes, Am. J. Physiol. Endocrinol. Metab. 283 (2002) E413–E422.
[24] Y. Kaburagi, S. Satoh, H. Tamemoto, R. Yamamoto-Honda, K. Tobe, K. Veki, T. Yamauchi, E. Kono-Sugita, H. Sekihara, S. Aizawa, S.W. Cushman, Y. Akanuma, Y. Yazaki, T. Kadowaki, Role of insulin receptor substrate-1 and pp60 in the regulation of insulin-induced glucose transport and GLUT4 translocation in primary adipocytes, J. Biol. Chem. 272 (1997) 25839–25844.
[25] G.I. Shulman, Unraveling the cellular mechanism of insulin resistance in humans: new insights from magnetic resonance spectroscopy, Physiology 19 (2004) 183–190.
[26] G.M. Reaven, Banting lecture 1988. Role of insulin resistance in human disease, Diabetes 37 (1988) 1595–1607.

Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 1593–1599

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Effects of thorough mastication on postprandial plasma glucose concentrations in nonobese Japanese subjects

Hidehiko Suzuki[a], Mitsuo Fukushima[b,c], Shigeru Okamoto[a], Osamu Takahashi[a], Takuro Shimbo[a], Takeshi Kurose[c], Yuichiro Yamada[c], Nobuya Inagaki[c], Yutaka Seino[c,d], Tsuguya Fukui[a,*]

[a]*Department of General Medicine and Clinical Epidemiology, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan*
[b]*Department of Health Informatics Research, Translational Research Informatics Center, Foundation for Biomedical Research and Innovation, Kobe 650-0047, Japan*
[c]*Department of Diabetes and Clinical Nutrition, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan*
[d]*Division of Diabetes and Clinical Nutrition, Kansai-Denryoku Hospital, Osaka 553-0003, Japan*

Received 4 December 2004; accepted 24 June 2005

Abstract

Thorough mastication has the potential to affect postprandial plasma glucose concentrations by improving digestibility and absorption of nutrients. To evaluate the effects of mastication on postprandial plasma glucose concentration, we compared usual and thorough mastication in subjects with normal glucose tolerance (NGT group, n = 16) and subjects predisposed to type 2 diabetes (first-degree relatives of type 2 diabetic patients, subjects with impaired glucose tolerance, and type 2 diabetic patients) (predisposed group, n = 10) in a crossover trial of 52 test meals. Plasma glucose and serum insulin concentrations were measured for 3 hours postprandially, and the insulinogenic index (the ratio of incremental serum insulin to plasma glucose concentration during the first 30 minutes after meal) was calculated. In the NGT group, thorough mastication reduced the postprandial plasma glucose concentration at 90 minutes (5.8 ± 0.3 vs 6.5 ± 0.4 mmol/L, $P < .05$) and 120 minutes (5.4 ± 0.2 vs 6.3 ± 0.4 mmol/L, $P < .05$) and the area under the curve (AUC) from −15 to 180 minutes (19.1 ± 0.6 vs 20.6 ± 0.8 [mmol/L] · h, $P < .05$) without an increase in the AUC for insulin. In the predisposed group, thorough mastication significantly augmented plasma glucose and serum insulin concentrations and the AUCs compared with usual mastication. Thorough mastication elicited a significantly higher insulinogenic index than usual mastication in the NGT group (205.0 ± 27.6 vs 145.6 ± 17.7 pmol/mmol, $P < .05$), whereas the predisposed group showed significantly less early-phase insulin secretion than the NGT group. In the NGT group the postprandial plasma glucose concentration upon thorough mastication of meal was significantly lower, most probably because of the potentiation of early-phase insulin secretion. In the subjects predisposed to type 2 diabetes, thorough mastication did not potentiate early-phase insulin secretion and elicited a higher postprandial plasma glucose concentration.

1. Introduction

Fletcherism, the practice of chewing food slowly and thoroughly as an aid to digestion [1], was advocated by the American dietician Horace Fletcher (1849-1919). He found that prolonged mastication both inhibited overeating and contributed to reduced food intake [2]. Under laboratory conditions, it has been found that when people enjoy softer food, they masticate less and bite with less vigor [3]. Fast food such as hamburgers is highly palatable by clever seasoning and flavoring, but soft and airy and with a generally homogenous consistency, and is now so commonplace worldwide [4] that the physiological importance of thorough mastication is barely recognized.

The major physiological function of mastication is the mechanical disruption of food into small particles suitable for gastrointestinal absorption of nutrients [5]. Preabsorptive or cephalic-phase insulin release, a vagally mediated response, occurs within the first few minutes of food ingestion [6] and is thought to be required for normal postprandial glucose tolerance [7]. Thus, mastication plays a crucial role in determining the postprandial plasma glucose concentration. Modified sham feeding, in which food is chewed and tasted but not swallowed [8], has been shown to elicit cephalic-phase insulin release [9], but few studies have examined the relation between thorough mastication and postprandial plasma glucose concentra-

* Corresponding author. Tel.: +81 75 751 4210; fax: +81 75 751 4211.
E-mail address: fkts@luke.or.jp (T. Fukui).

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.06.006

tions. Read et al [10] found that thoroughly masticating food rather than merely swallowing it increased plasma glucose concentrations after the ingestion of 4 kinds of carbohydrate (sweet corn, potato, rice, and apple) in 6 healthy subjects, mostly because of improved digestibility and absorption. However, no variables other than the plasma glucose concentration during the early postprandial period were examined. The aim of the present study is to evaluate the effect of thorough mastication on postprandial plasma glucose concentrations. We used a mixed-nutrient meal of hamburger steak and rice as the test meal. Hamburger steak is a kind of processed meat, the frequent consumption of which is reported to increase the risk of type 2 diabetes [11]. The hamburger steak and rice used in this study were both readily swallowed without thorough mastication.

2. Subjects and methods

2.1. Subjects

A total of 26 volunteers (17 men and 9 women; mean age, 38.9 ± 11.5 [SD] years [range, 25-71 years]; mean body mass index [BMI], 21.8 ± 2.8 kg/m^2 [range, 16.8-26.8 kg/m^2]) participated in the study. Sixteen had normal glucose tolerance (NGT), 6 were first-degree relatives of type 2 diabetic patients, 2 had impaired glucose tolerance (IGT), and 2 had mild type 2 diabetes mellitus without pharmacotherapy. None of the subjects were taking medication known to influence glucose concentration. Subjects were classified into 2 groups, one with NGT (NGT group) and the other with a predisposition to diabetes (predisposed group), which comprised IGT, mild type 2 diabetes, and first-degree relatives of type 2 diabetic patients, and underwent 1 session of each mastication procedure. Fourteen of the 16 NGT subjects and all 10 subjects in the predisposed group underwent 75-g oral glucose tolerance test (OGTT). Subjects with fasting plasma glucose (FPG) of less than 5.6 mmol/L and HbA_{1c} of less than 5.0% and/or with FPG of less than 6.1 mmol/L and 2-hour glucose of less than 7.8 mmol/L in OGTT by 1998 World Health Organization diagnostic criteria [12] were classified as NGT. IGT and type 2 diabetes also were defined according to World Health Organization criteria. Table 1 shows the clinical characteristics of the NGT and the predisposed groups. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, Kyoto University, and was conducted in accordance with the Declaration of Helsinki. All subjects gave written informed consent.

Table 1
Clinical characteristics of the 26 subjects

	NGT group	Predisposed group
n (male/female)	16 (9/7)	10 (8/2)
Definition	16 NGT	6 First-degree relatives, 2 IGT, 2 type 2 diabetes
Age (y)	35.6 ± 2.1	44.1 ± 4.4
BMI (kg/m^2)	21.2 ± 0.6	23.0 ± 1.0
HbA_{1c} (%)	4.6 ± 0.1	5.4 ± 0.4
FPG (mmol/L)	5.2 ± 0.1	5.8 ± 0.2
Fasting insulin (pmol/L)	57.4 ± 5.0	49.5 ± 4.3
Total cholesterol (mmol/L)	5.1 ± 0.2	5.0 ± 0.2
HDL cholesterol (mmol/L)	1.8 ± 0.1	1.5 ± 0.1
Triglyceride (mmol/L)	1.0 ± 0.1	1.0 ± 0.1

Data are means (of 2 sessions) ± SE. There were no significant differences between the NGT and the predisposed groups. HDL indicates high-density lipoprotein.

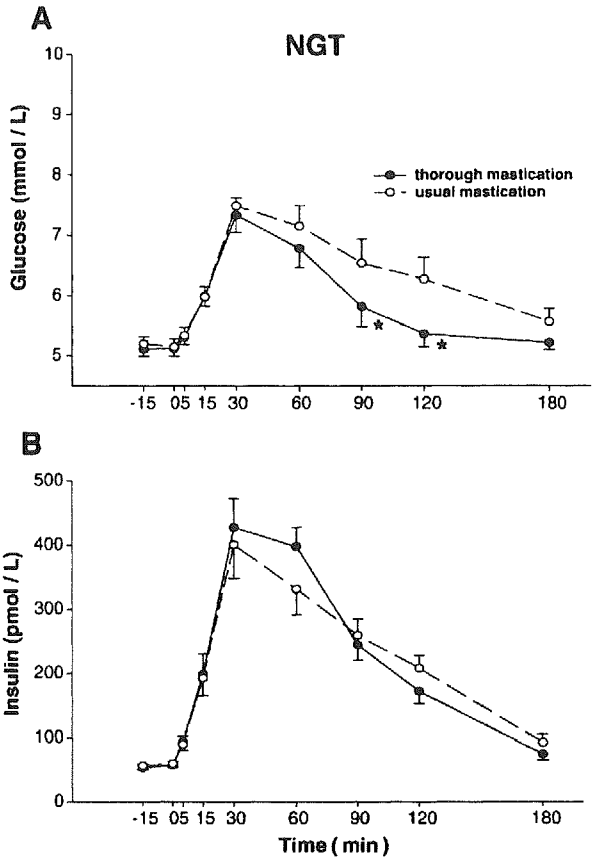

Fig. 1. Plasma glucose (A) and serum insulin (B) concentrations in the NGT group at various time points in usual and thorough mastication. Data are means ± SE, n = 16, *P < .05.

2.2. Experimental protocol

The study was a crossover experiment that included 52 sessions. After overnight fasting for at least 12 hours, the subjects began each eating session at 8:00 AM. A butterfly needle was inserted into an antecubital vein to draw blood samples at −15 minutes and was kept open by a slow drip of physiological saline. Immediately after a blood sample was drawn at 0 minute, the test meal of 130 g hamburger steak of 962 kJ (230 kcal) (Tokiwa Kanpo Pharmaceutical, Osaka, Japan) and 100 g rice of 649 kJ (155 kcal) (Hagoromo Foods, Shizuoka, Japan), with a total energy content of 1611 kJ (385 kcal) comprising 51%, 15%, and 34% carbohydrate, protein, and fat, respectively, began. Each

food item was sealed in a retort pouch and heated in a standard microwave oven for 2 minutes before the meal. The hamburger steak and rice were divided into 8 equal portions. Each subject underwent both mastication procedures. In the "usual mastication" sessions, the subjects took 16 teaspoonfuls of food, chewing each teaspoonful for 10 seconds before swallowing. In the "thorough mastication" sessions, each teaspoonful was swallowed only after 30 seconds of chewing. The rate of mastication was maintained at about 1 cycle per second in each session. Thus, thorough mastication involved 3-fold more bites than usual mastication. As the difference in the time taken eating might be a confounding factor, the subjects in usual mastication paused for 20 seconds after every 10 seconds, during which they were permitted to drink nonenergetic water, equalizing the duration of all meals at 8 minutes. The succession of mastication procedure was randomized for each subject. The average duration of the experiment was 9.9 days for male subjects. Female subjects participated only during the follicular phase of the menstrual cycle, with the interval fixed at 4 weeks to reduce variations in insulin sensitivity [13].

Blood samples for glucose and insulin were withdrawn at −15 and 0 minute before each meal and at 5, 15, 30, 60, 90, 120, and 180 minutes after each meal.

2.3. Analytical methods

Plasma glucose was measured by the glucose oxidase method using a Hitachi Automatic Analyzer 7170 (Hitachi, Tokyo, Japan). Serum insulin was measured in duplicate using LS regand Eiken insulin (Eiken, Tokyo, Japan) by automatic chemiluminescence enzyme immunoassay analyzer BCS 600 (SRL, Tokyo, Japan). The cross-reactivity to proinsulin, C-peptide, and split insulin was 0.01%, 0%, and 0%, respectively.

2.4. Data analysis

Values are expressed as mean ± SE unless otherwise noted. Statistical analysis was performed using StatView 5.0 (Abacus Concepts, Berkeley, CA). The area under the curve (AUC) was calculated according to the trapezoid rule. FPG and fasting serum insulin concentrations are the average of 2 premeal values (−15 and 0 minute). The insulinogenic index (II) [14], the ratio of the incremental serum insulin to plasma glucose concentration during the first 30 minutes after glucose ingestion calculated by OGTT (II_{OGTT}), has been commonly used as a measure of early-phase insulin secretion [15-18] since it was proposed by Seltzer et al [19] in 1967. In this study, the II during the first 30 minutes after meal tolerance test (MTT) (II_{MTT}) was calculated as the serum insulin concentration (30 − 0 minute)/plasma glucose concentration (30 − 0 minute) (pmol/mmol), and II_{MTT} and II_{OGTT} were compared. To estimate differences between 2 means, Student paired t test was performed with paired variates. To compare unpaired variates, Student unpaired t test with equal variances or Welch test with unequal variances was used. Multiple comparisons between differences among individual time points were done by analysis of variance (repeated measures) followed by Student t test with Bonferroni correction. Pearson r was used to evaluate univariate correlations. $P < .05$ was considered statistically significant.

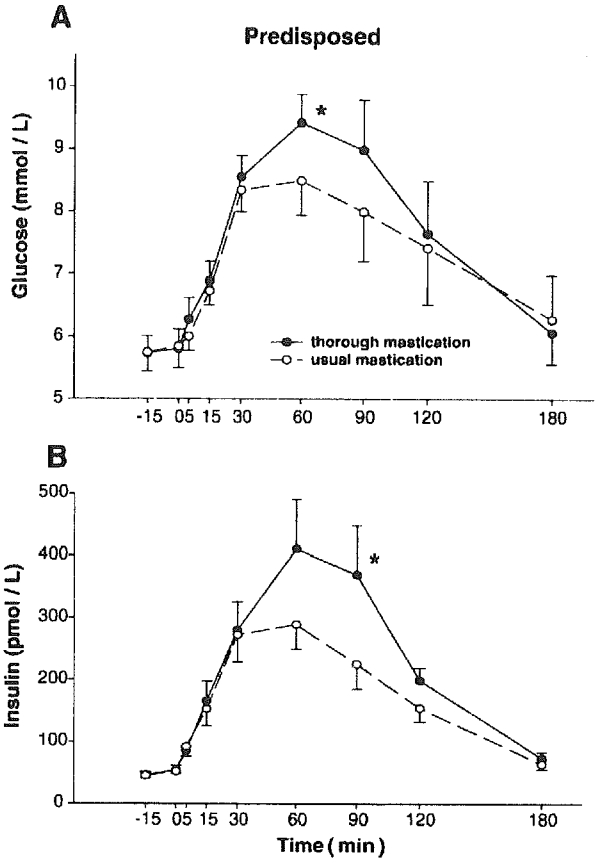

Fig. 2. Plasma glucose (A) and serum insulin (B) concentrations in the predisposed group at various time points in usual and thorough mastication. Data are means ± SE, n = 10. *$P < .05$.

Table 2
Comparison of the total AUCs (−15 to 180 minutes) for glucose and insulin in the NGT and the predisposed groups

	Glucose AUC ([mmol/L] · h) (−15 to 180 min)	Insulin AUC ([pmol/L] · h) (−15 to 180 min)
NGT group		
Usual mastication	20.6 ± 0.8	722.5 ± 60.3
Thorough mastication	19.1 ± 0.6*	722.5 ± 50.9
Predisposed group		
Usual mastication	23.7 ± 1.7	574.7 ± 65.3
Thorough mastication	24.9 ± 1.5**	755.5 ± 91.8**

Data are means ± SE.
* $P < .05$, significantly different from usual mastication in each group.
** $P < .01$, significantly different from usual mastication in each group.

3. Results

All 26 subjects completed all sessions of the test meals. Fig. 1 shows the plasma glucose and serum insulin concentrations in the NGT group in usual and thorough mastication. The plasma glucose concentration in both masticatory procedures increased in the first 30 minutes to 7.4 and 7.3 mmol/L, respectively. On the other hand, plasma glucose in thorough mastication decreased more rapidly than in usual mastication and was significantly reduced at 90 and 120 minutes (90 minutes, 5.8 ± 0.3 vs 6.5 ± 0.4 mmol/L, $P < .05$; 120 minutes, 5.4 ± 0.2 vs 6.3 ± 0.4 mmol/L, $P < .05$) (Fig. 1A). The AUC for glucose in the NGT group from −15 to 180 minutes was significantly less in thorough mastication than in usual mastication ($P = .017$) (Table 2). Insulin secretion was increased in thorough mastication from 5 minutes to nearly 90 minutes (the major difference occurring at 60 minutes: 397.5 ± 29.4 vs 332.2 ± 39.5 pmol/L) (Fig. 1B). The AUC for insulin in the NGT group from 90 to 180 minutes was significantly less in thorough mastication than in usual mastication (228.9 ± 20.1 vs 268.3 ± 23.0 [pmol/L] · h, $P = .044$). The total AUCs for insulin in the 2 mastication procedures were the same (Table 2).

The data on the predisposed group are shown in Fig. 2. During the first 30 minutes, both plasma glucose and serum insulin concentrations showed a similar pattern in the 2 mastication procedures. At 60 minutes, there was a significantly higher glucose response in thorough mastication than in usual mastication (9.4 ± 0.45 vs 8.4 ± 0.55 mmol/L, $P < .05$) (A), as well as a significantly higher insulin response at 90 minutes (370.9 ± 78.9 vs 226.0 ± 40.2 pmol/L, $P < .05$) (B). The AUCs for both glucose ($P = .008$) and insulin ($P = .002$) in the predisposed group were increased significantly in thorough mastication compared with usual mastication (Table 2).

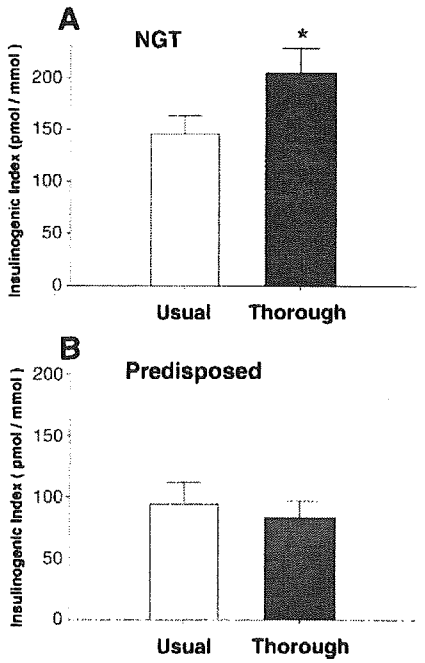

Fig. 3. II_{MTT} in the NGT group (n = 16) (A) and the predisposed group (n = 10) (B) during the first 30 minutes after meal in usual and thorough mastication. Data are means ± SE, $*P < .05$.

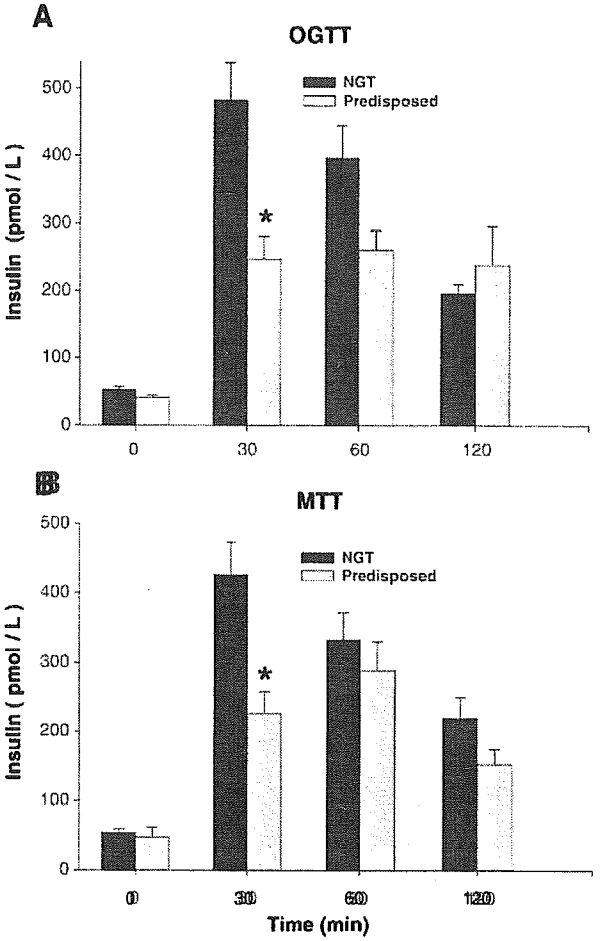

Fig. 4. Serum insulin concentrations in the NGT group (black bars, n = 14) and the predisposed group (gray bars, n = 10) measured by OGTT (A) and MTT in usual mastication (B) at 4 time points. Data are means ± SE, $*P < .05$.

Fig. 3 shows the II measured by MTT for the 2 mastication procedures in the NGT (A) and the predisposed group (B). In the NGT group, II_{MTT} in thorough mastication was significantly higher than in usual mastication (205.0 ± 27.6 vs 145.6 ± 17.7 pmol/mmol, $P = .02$) (Fig. 3A). On the other hand, there was no significant difference in II_{MTT} between the 2 mastication procedures in the predisposed group (B).

Fig. 4 shows the serum insulin concentrations at 4 time points in OGTT (A) and MTT in usual mastication (B) in the NGT group (n = 14) and the predisposed group (n = 10) in subjects who underwent both OGTT and MTT. The

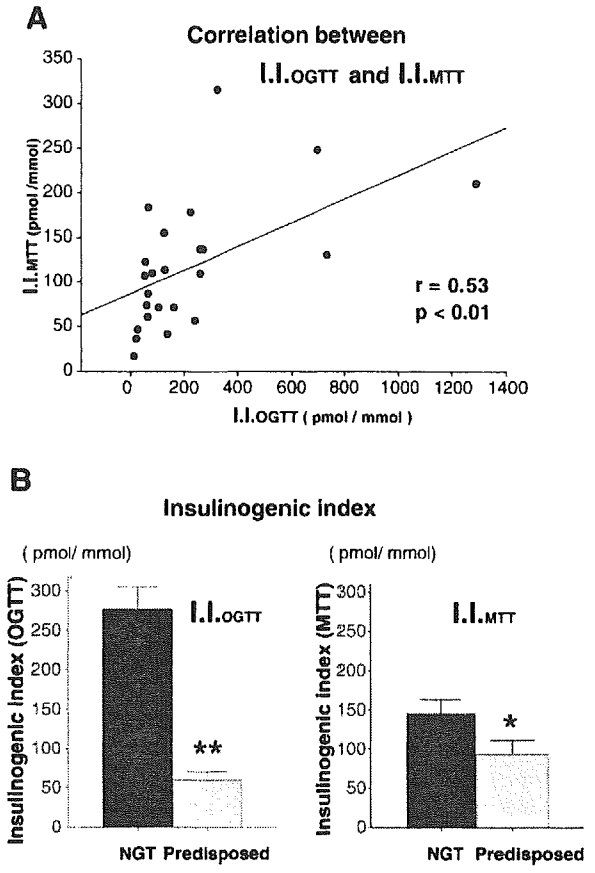

Fig. 5. A, Correlation between the II estimated from OGTT and MTT in usual mastication in 24 subjects who underwent both OGTT and MTT. B, II_{OGTT} (II during the first 30 minutes after OGTT) and II_{MTT} (II during the first 30 minutes after MTT) in usual mastication in the NGT group (n = 14) and the predisposed group (n = 10). Data are means ± SE, $^{*}P < .05$, $^{**}P < .01$.

serum insulin concentrations in the NGT and the predisposed groups showed a general similarity in both OGTT and MTT. The fasting serum insulin concentrations in both groups were similar in both tests. The peak serum insulin concentration occurred at 30 minutes in the NGT group and at 60 minutes in the predisposed group in both tests. The serum insulin concentration at 30 minutes was significantly higher in the NGT group than in the predisposed group in both tests (OGTT, 482.9 ± 55.2 vs 246.8 ± 34.4 pmol/L, $P < .05$; MTT, 426.2 ± 48.1 vs 227.4 ± 30.9 pmol/L, $P < .05$). There was no significant difference in the serum insulin concentration between the 2 groups at 60 and 120 minutes in both tests.

Fig. 5 shows the correlation between II_{OGTT} and II_{MTT} in usual mastication (A) and the IIs (B). II_{MTT} was significantly correlated with II_{OGTT} in the 24 subjects who underwent both tests ($r = 0.53$, $P < .01$) (A). II_{OGTT} (276.6 ± 28.0 vs 60.1 ± 11.3 pmol/mmol, $P = .004$) and II_{MTT} (145.8 ± 17.8 vs 94.0 ± 17.6 pmol/mmol, $P = .046$) were significantly higher in the NGT group than in the predisposed group (B).

4. Discussion

In this study, we compared the effects of thorough mastication on postprandial glucose and insulin secretion in subjects with NGT and subjects predisposed to type 2 diabetes. Thorough mastication was especially effective in reducing the postprandial plasma glucose concentrations in the NGT group, probably because of greater early-phase insulin secretion.

Surprisingly, the AUC for glucose was significantly less in thorough mastication compared with usual mastication, without an increase in the AUC for insulin. Mastication breaks food into small pieces, stimulates salivation, and mixes food with salivary enzymes, improving hydrolysis of carbohydrates in the mouth and stomach [10] and enhancing glycemic and insulinemic responses. Thus, thorough mastication should be expected to increase both postprandial plasma glucose and serum insulin concentrations. However, regardless of the mastication procedure, the plasma glucose and serum insulin concentration reached a peak at 30 minutes in the NGT group. In addition, II_{MTT} in thorough mastication was significantly higher than in usual mastication. Apparently, NGT subjects have sufficient early-phase insulin secretory capacity to lower the plasma glucose concentration after the more rapid absorption of glucose in thorough mastication. Thorough mastication was especially effective in NGT subjects in potentiating insulin secretion from 5 minutes (the cephalic-phase) to 90 minutes, resulting in lower plasma glucose concentrations after 30 minutes. Insulin secretion from 90 to 180 minutes was thus reduced, resulting in the same AUC for insulin in the 2 mastication procedures in the NGT group. Thus, the present study suggests that people with NGT can reduce postprandial plasma glucose concentrations by masticating food thoroughly.

In contrast to the NGT group, thorough mastication was not effective in reducing the postprandial plasma glucose concentration in the predisposed group. Compared with usual mastication, the glycemic response in thorough mastication was significantly enhanced at 60 minutes, and the insulinemic response was enhanced at 90 minutes. In addition, the AUCs for both glucose and insulin in thorough mastication were significantly greater than in usual mastication. In addition, in contrast to the NGT group, there was no significant difference in the II between the 2 mastication procedures in the predisposed group. The fact that thorough mastication did not potentiate insulin secretion in the predisposed group during the first 30 minutes suggests inability of the beta-cells to respond promptly to glucose stimulation. Thorough mastication might be expected to promote satiation with reduced food intake in ordinary life. Food intake can be reduced by a number of monoamines acting on noradrenaline, serotonin, dopamine, and histamine receptors within the hypothalamus [20]. The rate of 40 masticating cycles per minute has been shown to increase the firing rate of serotonergic neurons in cats [21]. Moreover, thorough mastication enhances satiation

independently of energy expenditure by activating neuronal histamine in the hypothalamus [22]. Accordingly, thoroughly masticating food might also benefit similarly predisposed individuals in daily life.

Early-phase insulin secretion is known to be disturbed in patients with type 2 diabetes, IGT, and normoglycemic first-degree relatives of patients with type 2 diabetes [14,23-27], so first-degree relatives of type 2 diabetic patients were included in the predisposed group. Early-phase insulin secretion in both OGTT and MTT was significantly less in the predisposed group than in the NGT group. Thus, the correlation between II_{MTT} and II_{OGTT} observed in the present study suggests that II_{MTT} calculated by the same formula as II_{OGTT} can be used as an index of early-phase insulin secretion. The II_{MTT} in the NGT and the predisposed groups of the present study were clearly different, most probably because of the difference in early-phase insulin secretion, which may underlie the altered postprandial plasma glucose concentrations. Early-phase insulin secretion is commonly referred to in both OGTT and MTT analyses [15-18]. Although the relation between the first-phase insulin response to intravenous glucose challenge and the early insulin response to oral glucose has been investigated recently [28-30], further studies are required to distinguish first- and second-phase insulin secretion sufficiently for in vivo comparison of mastication procedures. Because the plasma glucose concentrations increased gradually in this study, we used the term *early-phase insulin secretion*.

Thorough mastication was found to reduce the postprandial plasma glucose concentration mainly in the NGT group. Although the most important substance in physiological regulation of insulin release is glucose, incretin hormones (gastric inhibitory peptide [GIP] and glucagon-like peptide 1 [GLP-1]) also play important roles in postprandial insulin secretion in healthy subjects [7]. GIP and GLP-1 are released from the gut to the portal vein and are diluted when entering the systemic circulation. Only 10% to 15% of GLP-1 reaches systemic circulation and the pancreas in the intact form [31,32]. In the present study, thorough mastication elicited at most a 1.2-fold increase in the peripheral serum insulin concentration at 60 minutes in the NGT group compared with usual mastication. In a rodent study, intraportal injection of a pharmacological dose of GLP-1 was reported to evoke a peak of only a 2-fold increase in the peripheral insulin response to portal glucose compared with the control condition [33]. Accordingly, the slight change in GIP and GLP-1 in systemic circulation that may correspond to the difference in peripheral serum insulin concentrations in the NGT group would be difficult to detect by peripheral blood sampling. Further studies are required to determine whether differences in the rate of mastication affect incretin concentrations. In addition, other hormones, including glucagon, growth hormone, and cortisol, and other nutrients (amino acids) are also involved in insulin secretion upon meal ingestion [30,34]. We also measured the plasma arginine concentration. Insulin secretion is stimulated by amino acids after the digestion of protein in meal [35]. The AUC of incremental arginine from 0 to 120 minutes in thorough mastication was significantly greater than in usual mastication (data not shown). Thus, increased absorption of arginine in thorough mastication is at least partly responsible for the increased insulin secretion in NGT subjects.

The pancreas has rich innervation from both the sympathetic and parasympathetic nervous system. Sympathetic fibers are found primarily in the splanchnic nerve, whereas parasympathetic fibers are found in the vagus nerve [36]. According to the study of Rasmussen et al [37] and our previous report [38], neural factors play important roles in the normal pattern of insulin secretion. There are 2 neural stages before the late enteric stage when nutrients are absorbed [37]. During the cephalic phase in thorough mastication, the release of acetylcholine is considered to be stimulated more strongly than in usual mastication through activation of vagal-efferent fibers. Thus, during the early enteric phase, when the neurons of the enteric nervous system are activated by nutrients entering the intestine [37], thorough mastication may promote stronger release of cholecystokinin by augmenting gastric emptying. Accordingly, acetylcholine and cholecystokinin might contribute to potentiating early-phase insulin secretion in thorough mastication in the NGT group. In contrast, in the predisposed group, potentiation of early-phase insulin secretion in thorough mastication was not observed, most likely because of the poor response of the beta-cell to neural stimulation.

In addition, mastication mixes food particles with saliva. In studies comparing normal and diabetic subjects, the flow rate of saliva, the volume of saliva secreted per minute, is diminished significantly in diabetic patients [39]. Diabetic neuropathy may well account for this decrease [40]. Because the concentration of amylase in diabetic subjects has been reported to be lower [40], higher [39], and similar [41] to healthy subjects, whether improved exposure of food particles to amylase in saliva affects postprandial plasma glucose concentrations in persons with NGT remains to be determined.

Although the predisposed group was composed of 3 subgroups, first-degree relatives of type 2 diabetic patients, IGT, and type 2 diabetes, it was clearly distinguished from the NGT group in terms of early-phase insulin secretion. However, further investigation of the effect of thorough mastication in each of the subgroups with more samples would be informative.

In conclusion, in the present study, thorough mastication elicited lower postprandial plasma glucose concentrations than usual mastication in the NGT group, most probably because of the potentiation of early-phase insulin secretion. In contrast, in the predisposed group, thorough mastication did not potentiate early-phase insulin secretion and elicited higher postprandial plasma glucose concentrations.

Acknowledgment

This study was supported in part by the Japan Arteriosclerosis Prevention Fund (JAPF).

We thank Tokiwa Kanpo Pharmaceutical, SRL, Use Techno, and Abbott Japan for assistance in this study.

References

[1] Guralink D, editor. Webster's New World dictionary. New York (NY): Simon & Schuster; 1984. p. 533.
[2] Christen AG, Christen JA. Horace Fletcher (1849-1919): "the great masticator". J Hist Dent 1997;45:95-100.
[3] Bellisle F, Guy-Grand B, Le Magnen J. Chewing and swallowing as indices of the stimulation to eat during meals in humans: effects revealed by the edogram method and video recordings. Neurosci Biobehav Rev 2000;24:223-8.
[4] Schlosser E. Fast food nation; the dark side of the all-American meal. New York (NY): HarperCollins; 2002.
[5] Pedersen AM, Bardow A, Jensen SB, et al. Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion. Oral Dis 2002;8:117-29.
[6] Teff KL, Townsend RR. Early phase insulin infusion and muscarinic blockade in obese and lean subjects. Am J Physiol 1999; 277:R198-208.
[7] Ahrén B, Holst JJ. The cephalic insulin response to meal ingestion in humans is dependent on both cholinergic and noncholinergic mechanisms and is important for postprandial glycemia. Diabetes 2001;50:1030-8.
[8] Robertson MD, Jackson KG, Williams CM, et al. Prolonged effects of modified sham feeding on energy substrate mobilization. Am J Clin Nutr 2001;73:111-7.
[9] Teff KL, Levin BE, Engelman K. Oral sensory stimulation in men: effects on insulin, C-peptide, and catecholamines. Am J Physiol 1993;265:R1223-30.
[10] Read NW, Welch IM, Austen CJ, et al. Swallowing food without chewing; a simple way to reduce postprandial glycaemia. Br J Nutr 1986;55:43-7.
[11] Van Dam RM, Willet WC, Rimm EB, et al. Dietary fat and meat intake in relation to risk of type 2 diabetes in men. Diabetes Care 2002;25:417-24.
[12] Alberti KGMM, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539-53.
[13] González-Ortiz M, Martínez-Abundis E, Lifshitz A. Insulin sensitivity and sex steroid hormone levels during the menstrual cycle in healthy women with non–insulin-dependent diabetic parents. Gynecol Obstet Invest 1998;46:187-90.
[14] Seino Y, Ikeda M, Yawata M, et al. The insulinogenic index in secondary diabetes. Horm Metab Res 1975;7:107-15.
[15] Kahn SE. The importance of the β-cell in the pathogenesis of type 2 diabetes mellitus. Am J Med 2000;108:2S-8S.
[16] Kosaka K, Hagura R, Kuzuya T. Insulin responses in equivocal and definite diabetes, with special reference to subjects who had mild glucose intolerance but later developed definite diabetes. Diabetes 1977;26:944-52.
[17] Yoneda H, Ikegami H, Yamamoto Y, et al. Analysis of early-phase insulin responses in nonobese subjects with mild glucose intolerance. Diabetes Care 1992;15:1517-21.
[18] Caumo A, Luzi L. First-phase insulin secretion: does it exist in real life? Considerations on shape and function. Am J Physiol Endocrinol Metab 2004;287:E371-85.
[19] Seltzer HS, Allen EW, Herron Jr AL, et al. Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. J Clin Invest 1967;46:323-35.
[20] Bray GA. A concise review on the therapeutics of obesity. Nutrition 2000;16:953-60.
[21] Lewkowski MD, Barr RG, Sherrard A, et al. Effects of chewing gum on responses to routine painful procedures in children. Physiol Behav 2003;79:257-65.
[22] Sakata T, Yoshimatsu H, Kurokawa M. Hypothalamic neuronal histamine: implications of its homeostatic control of energy metabolism. Nutrition 1997;13:403-11.
[23] Fukushima M, Usami M, Ikeda M, et al. Insulin secretion and insulin sensitivity at different stages of glucose tolerance: a cross-sectional study of Japanese type 2 diabetes. Metabolism 2004;53: 831-5.
[24] Calles-Escandon J, Robbins DC. Loss of early phase of insulin release in humans impairs glucose tolerance and blunts thermic effect of glucose. Diabetes 1987;36:1167-72.
[25] Meier JJ, Hücking K, Holst JJ, et al. Reduced insulinotropic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. Diabetes 2001;50:2497-504.
[26] Kahn SE, Prigeon RL, Schwartz RS, et al. Obesity, body fat distribution, insulin sensitivity and islet β-cell function as explanations for metabolic diversity. J Nutr 2001;131:354S-60S.
[27] Suzuki H, Fukushima M, Usami M, et al. Factors responsible for development from normal glucose tolerance to isolated postchallenge hyperglycemia. Diabetes Care 2003;26:1211-5.
[28] Stumvoll M, Mitrakou A, Pimenta W, et al. Use of the oral glucose tolerance test to assess insulin release and insulin sensitivity. Diabetes Care 2000;23:295-301.
[29] Bonadonna RC, Stumvoll M, Fritsche A, et al. Altered homeostatic adaptation of first- and second phase β-cell secretion in the offspring of patients with type 2 diabetes; studies with a minimal model to assess β-cell function. Diabetes 2003;52:470-80.
[30] Basu R, Breda E, Oberg AL, et al. Mechanisms of the age-associated deterioration in glucose tolerance; contribution of alterations in insulin secretion, action, and clearance. Diabetes 2003;52:1738-48.
[31] Hansen L, Deacon CF, Ørskov C, et al. Glucagon-like peptide-1-(7-36) amide is transformed to glucagon-like peptide-1-(9-36) amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. Endocrinology 1999;140:5356-63.
[32] Holst JJ, Ørskov C. The incretin approach for diabetes treatment; modulation of islet hormone release by GLP-1 agonism. Diabetes 2004;53(Suppl 3):S197-S204.
[33] Balkan B, Li X. Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms. Am J Physiol Regul Integr Comp Physiol 2000;279:R1449-54.
[34] Henquin JC, Ravier MA, Nenquin M, et al. Hierarchy of the β-cell signals controlling insulin secretion. Eur J Clin Invest 2003;33: 742-50.
[35] Genuth SM. The endocrine system. In: Robert MB, Matthew NL, editors. Physiology. 4th ed. St. Louis (Mo): Mosby; 1998. p. 800-46.
[36] Teff K. Nutritional implications of the cephalic-phase reflexes: endocrine responses. Appetite 2000;34:206-13.
[37] Rasmussen H, Zawalich KC, Ganesan S, et al. Physiology and pathophysiology of insulin secretion. Diabetes Care 1990;13:655-66.
[38] Kurose T, Seino Y, Nishi S, et al. Mechanism of sympathetic neural regulation of insulin, somatostatin, and glucagon secretion. Am J Physiol 1990;258:E220-7.
[39] López ME, Colloca ME, Páez RG, et al. Salivary characteristics of diabetic children. Braz Dent J 2003;14:26-31.
[40] Newrick PG, Bowman C, Green D, et al. Parotid salivary secretion in diabetic autonomic neuropathy. J Diabetes Complications 1991;5: 35-7.
[41] Ben-Aryeh H, Serouya R, Kanter Y, et al. Oral health and salivary composition in diabetic patients. J Diabetes Complications 1993; 7:57-62.

Y. Kawasaki[1]
A. Taniguchi[2]
M. Fukushima[3]
Y. Nakai[4]
A. Kuroe[2]
M. Ohya[2]
S. Nagasaka[5]
Y. Yamada[1]
N. Inagaki[1]
Y. Seino[1,2]

Soluble TNF Receptors and Albuminuria in Non-obese Japanese Type 2 Diabetic Patients

Abstract

The aim of this study was to investigate the relationships between albuminuria and tumor necrosis factor (TNF)-α or soluble TNF receptors (sTNF-R1, sTNF-R2) in eighty-eight non-obese Japanese type 2 diabetic patients stratified into two groups according to albuminuria status-microalbuminuria or normoalbuminuria. Patients with microalbuminuria were older and had significantly higher concentrations of sTNF-R1 and sTNF-R2 than those with normoalbuminuria. There was, however, no significant difference in sex, diabetes duration, smoking, BMI, systolic and diastolic blood pressure, HbA_{1c}, serum creatinine, and lipid profile between the two groups. Although serum TNF-α was positively correlated to serum sTNF-R1 and sTNF-R2, serum TNF-α level did not differ with respect to albuminuria. Univariate regression analysis showed that urinary albumin concentration was positively correlated to age ($r = 0.380$, $p < 0.001$), serum creatinine ($r = 0.214$, $p < 0.05$) and concentrations of sTNF-R1 ($r = 0.364$, $p < 0.001$) and sTNF-R2 ($r = 0.342$, $p < 0.005$). Other variables, including TNF-α, were not associated with albuminuria. Multiple regression analyses showed that urinary albumin concentration was independently predicted by the level of sTNF-R1 ($F = 32.1$), which explained 26.3% of the variability of urinary albumin concentration. From these results, it can be concluded that serum soluble TNF receptor is an important independent factor associated with albuminuria in non-obese Japanese type 2 diabetic patients.

Key words
sTNF receptors · TNF-alpha · Albuminuria · Diabetes

Introduction

The major clinical consequence of type 2 diabetes is mortality and morbidity from atherosclerotic vascular disease, especially coronary heart disease (CHD). The risk of CHD appears to be similar in patients with type 2 diabetes and impaired glucose tolerance [1,2]. Thus, factors other than the level of glycemia seem to accelerate the development of CHD in type 2 diabetes. This idea is supported by the observation that duration of diabetes and level of glycemia are not risk factors for atherosclerosis including CHD in type 2 diabetic patients [3,4]. Atherosclerosis can be evaluated by urinary albumin excretion rate. Increased urinary albumin excretion rate is not only used as an index of diabetic nephropathy but also as an independent risk factor for atherosclerosis, including cardiac disease, in type 2 diabetic patients. An association between microalbuminuria and cardiac disease in type 2 diabetic patients has been demonstrated [5].

A number of studies published have shown that age, smoking, blood pressure, blood glucose, and abnormalities in lipoprotein

Affiliation
[1] Department of Metabolism and Clinical Nutrition, Graduate School of Medicine, Kyoto University, Kyoto, Japan
[2] Division of Diabetes and Clinical Nutrition, Kansai-Denryoku Hospital, Osaka, Japan
[3] Department of Health Informatics Research, Translational Research Informatics Center, Foundation for Biochemical Research and Innovation, Kobe, Japan
[4] School of Health Sciences Faculty of Medicine, Kyoto University, Kyoto, Japan
[5] Division of Endocrinology and Metabolism, Jichi Medical School, Tochigi, Japan

Correspondence
Ataru Taniguchi, M.D. · Division of Diabetes and Clinical Nutrition · Kansai-Denryoku Hospital · 2-1-7 Fukushima · Fukushima-ku · Osaka-city · Osaka 553-0003 · Japan · Fax: +81 (6) 64 58-69 94 · E-Mail: K-58403@kepco.co.jp

Received 17 December 2004 · **Accepted after revision** 7 April 2005

Bibliography
Horm Metab Res 2005; 37: 617–621 © Georg Thieme Verlag KG Stuttgart · New York · DOI 10.1055/s-870536 · ISSN 0018-5043

particles may lead to the development of microalbuminuria. Inflammation has also been implicated in the evolution of albuminuria. Festa et al. [6] recently demonstrated that diabetic patients with microalbuminuria had significantly higher levels of C-reactive protein (CRP) and fibrinogen compared to those with normoalbuminuria. Both CRP and fibrinogen are considered to be inflammatory markers. Stehouwer et al. [7] also documented that increased urinary albumin excretion, endothelial dysfunction, and chronic inflammation are interrelated processes that develop in parallel, progress with time, and are strongly and independently associated with risk of death in type 2 diabetic patients.

Tumor necrosis factor (TNF) is a potent proinflammatory cytokine involved in the pathogenesis of atherosclerosis. TNF is known to induce a cascade of inflammatory reactions involving production of other cytokines, thus participating in the development of atherosclerosis. TNF binds two receptors so far identified referred to as TNF-R1 and TNF-R2. Both of these receptors exist in soluble forms. The two receptors share almost no homology outside the ligand binding domain, suggesting that they signal for different biological functions [8–10]. Elevated soluble TNF-R1 levels have recently been shown to be predictive of cardiovascular mortality in patients with chronic heart failure [11]. However, the studies investigating the relationship between soluble TNF receptors and vascular complications in diabetic patients are limited. Zoppini et al. [12] very recently demonstrated that soluble TNF-R1 level is higher in type 1 diabetic patients with microalbuminuria than those with normoalbuminuria. To the best of our knowledge, however, the relationships between urinary albumin excretion rate and the levels of the two soluble TNF receptors have not yet been examined in type 2 diabetic patients.

In this context, a major problem is that albuminuria itself has been associated with atherosclerotic vascular diseases such as renal failure, cerebral infarction and CHD. Moreover, overweight condition or hyperglycemia *per se* may affect albuminuria, TNF-α, and soluble TNF receptors concentrations in humans [13,14]. We therefore recruited non-obese, well-controlled unique Japanese type 2 diabetic patients without evidence of vascular complications including CHD, cerebral infarction, or renal failure, taking into account of body mass index and fasting glucose level. This is the first finding that albuminuria is independently associated with serum level of soluble TNF receptor in non-obese well-controlled unique Japanese type 2 diabetic patients.

Subjects and Methods

After informed consent was obtained, forty-five diabetic patients with microalbuminuria (twenty-nine men and sixteen women) and forty-three patients with normoalbuminuria (thirty-four men and nine women) were enrolled in the present study. They all were non-obese (BMI < 27 kg/m^2) Japanese type 2 diabetic patients [15]. Type 2 diabetes mellitus was diagnosed based on the WHO criteria [16]. All subjects had ingested at least 150 g of carbohydrate in the three days before the study. Forty-one patients (91%) with microalbuminuria and 37 patients (86%) with normoalbuminuria were taking sulfonylureas, respectively. The rests were treated by diet alone. They all have not received insulin therapy. Blood pressure was measured according to a standard procedure and hypertension was defined as systolic blood pressure ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg or current use of antihypertensive medication. Twenty-two patients (49%) (Ca antagonist 11, ACE-I 8, ARB 3) with microalbuminuria and fifteen patients (35%) (Ca antagonist 5, ACE-I 6, both 2, ARB 2) with normoalbuminuria were treated with antihypertensive drugs, respectively. Fourteen patients (31%) with microalbuminuria and seventeen patients (40%) with normoalbuminuria were treated with lipid lowering agents, respectively. Cigarette smoking was dichotomized into "never" and "ever" (including past and current) using a questionnaire. There was no significant difference in gender, smoking, or medication status between the patients with microalbuminuria and those with normoalbuminuria (Table **1**). They did not consume alcohol or perform heavy exercise for at least one week before the study.

Blood was drawn in the morning after a 12 h fast. Plasma glucose was measured using the glucose oxidase method. Triglycerides, total cholesterol, and HDL cholesterol were also measured. The LDL cholesterol level was calculated using the Friedewald formula [17]. Serum insulin was measured using a two-site immunoradiometric assay (Insulin Riabead II, Dainabot, Japan). Coefficients of variation were 4% for insulin > 25 μU/ml and 7% for insulin < 25 μU/ml, respectively. Serum TNF-α concentrations were measured by enzyme immunoassay kit (Quantikine HS Human TNF-α immunoassay kit, R&D systems. Inc, Minneapolis, MN, USA) and serum concentrations of sTNF-R1 and sTNF-R2 were measured by enzyme-linked immunosorbent assay (ELISA; BIOTRAK, Amersham Life Sciences, Uppsala, Sweden) as described previously [18]. The limits of sensitivity for TNF-α, sTNF-R1 and sTNF-R2 were 0.5 pg/ml, 25 pg/ml and 50 pg/ml, respectively.

Urinary albumin concentration was assessed in a morning spot urine sample using a commercial enzymatic immunoassay. Urinary albumin concentration was measured in duplicate and the mean of the two values was used for the study. Intraassay and interassay coefficients of variation were less than 7% (Orion, Espoo, Finland). Several reports have indicated that early morning spot urine is usually sufficient for detecting the presence of microalbuminuria [19,20]. In the present study, we calculated urinary albumin excretion rate as a ratio of urinary albumin and urinary creatinine that markedly enhances the accuracy of the single spot urine sample in the assessment of microalbuminuria [21]. Microalbumiuria was defined as a urinary albumin concentration greater than 30 mg/g creatinine but less than 300 mg/g creatinine. Normoalbuminuria was defined as urinary albumin concentration less than 30 mg/g creatinine [22].

Statistical analysis

The statistical analysis was performed using the StatView 5 system (Statview, Berkeley, CA). The differences of mean were determined by the Mann-Whitney U-test. Data were expressed as the mean ± SEM. Simple (Spearman's rank) correlation coefficients between urinary albumin concentration and measures of variables were calculated, and a stepwise multiple regression analysis was then used to evaluate the independent association of these variables with urinary albumin concentration. Values of $p < 0.05$ were considered significant. In multivariate analysis, $F \geq 4$ was considered significant.

Table 1 Characteristics of patients with type 2 diabetes stratified by albuminuria status

	Microalbuminuria	*Normoalbuminuria*	*p*
Urinary albumin (mg/gCr)	90 ± 8	10 ± 1	0.001
Number of subjects	45	43	
M/F	29/16	34/9	0.159
Age (yrs)	65.6 ± 1.2	59.8 ± 1.3	0.001
Systolic blood pressure (mm Hg)	140 ± 3	133 ± 3	0.940
Diastolic blood pressure (mm Hg)	81 ± 2	83 ± 2	0.570
Smoking (yes/no)	9/36	13/30	0.328
Duration of diabetes (yrs)	11.7 ± 1.1	10.2 ± 1.1	0.314
BMI (kg/m^2)	22.6 ± 0.3	23.1 ± 0.3	0.301
Fasting glucose (mg/dl)	144 ± 3	138 ± 4	0.256
HbA_{1c} (%)	7.1 ± 0.1	7.0 ± 0.2	0.654
Fasting insulin (μU/ml)	6.6 ± 0.6	6.5 ± 0.4	0.540
Triglycerides (mg/dl)	119 ± 8	124 ± 9	0.705
Total cholesterol (mg/dl)	204 ± 6	204 ± 5	0.929
HDL cholesterol (mg/dl)	58 ± 2	59 ± 2	0.715
LDL cholesterol (mg/dl)	126 ± 5	126 ± 5	0.984
Serum creatinine (mg/dl)	0.77 ± 0.03	0.75 ± 0.02	0.537
TNF-α (ng/l)	3.5 ± 0.4	3.2 ± 0.2	0.479
sTNF-R1 (ng/l)	1272 ± 71	1084 ± 33	0.018
sTNF-R2 (ng/ml)	2172 ± 91	1933 ± 49	0.022
SU/diet	40/5	35/8	0.094
HMG-CoA reductase inhibitor	7/38	5/38	0.284
Bezafibrate	7/38	12/31	0.090
Ca antagonist	11/34	7/36	0.147
ACE inhibitor or ARB	8/37	8/35	0.500

Results

The clinical characteristics and clinical profile between the patients with microalbuminuria (n = 45) and normoalbuminuria (n = 43) were compared (Table **1**). Urinary albumin concentrations in patients with microalbuminuria and normoalbuminuria were 90 ± 8 (range, 35 – 282) and 10 ± 1 (range, 0.6 – 24.9) mg/g creatinine, respectively. There was no overlap in the urinary concentration of albumin between the two groups. While age was significantly greater in the patients with microalbuminuria than those with normoalbuminuria, no significant difference was observed in systolic and diastolic blood pressure, smoking, diabetes duration, BMI, fasting glucose, hemoglobin A_{1c}, or fasting insulin levels between the two groups. The two groups did not differ with respect to concentrations of serum triglycerides, total, HDL, or LDL cholesterol. Although there was no significant difference in the levels of serum creatinine and TNF-α, soluble TNF-R1 (1,272 ± 71 *vs.* 1,084 ± 33 pg/ml, p = 0.018), and soluble TNF-R2 (2172 ± 91 *vs.* 1933 ± 49 pg/ml, p = 0.022) were significantly higher in patients with microalbuminuria compared to those with normoalbuminuria.

Spearman's rank correlations of urinary albumin concentration with measures of variables were calculated for all our diabetic patients (Table **2**). Urinary albumin concentration was positively correlated with soluble TNF-R1 (r = 0.364, p < 0.001), soluble TNF-R2 (r = 0.342, p < 0.005), age (r = 0.380, p < 0.001), and serum creatinine (r = 0.214, p < 0.05). Other variables including systolic and diastolic blood pressure, TNF-α, and serum lipid profile including triglycerides were not associated with urinary albumin level. Multiple regression analyses were carried out using the stepwise procedure.

The analysis included urinary albumin level as a dependent variable and candidate risk factors (soluble TNF-R1, soluble TNF-R2, age, serum creatinine) as independent variables (Table **2**). The concentration of urinary albumin was independently predicted by serum concentration of soluble TNF-R1, which explained 26.3% of the variability of urinary albumin concentration in our patients. Other variables including age, serum creatinine, and soluble TNF-R2 were not independently associated with urinary albumin concentration in our non-obese Japanese type 2 diabetic patients. On the other hand, in a model incorporating BMI and systolic blood pressure, soluble TNF-R1 was also independently associated with urinary albumin concentration in our patients (Table **3**).

Discussion and Conclusions

This is the first published observation that soluble TNF-R1 is independently associated with urinary albumin concentration in non-obese Japanese type 2 diabetic patients.

Diabetic nephropathy has rapidly become an important public health problem since it is the leading cause of dialysis in Japan. Early detection of risk factors causing diabetic nephropathy before advanced renal damage occurs is therefore an urgent prior-

Table 2 Correlation of urinary albumin concentration to measures for variables in diabetic patients

	Univariate		*Multivariate*
	r	*p*	*F*
TNF-α	0.127	0.236	–
sTNF-R1	0.364	< 0.001	32.1
sTNF-R2	0.342	< 0.005	0.2
Age	0.380	< 0.001	1.9
Serum creatinine	0.214	0.046	0.1
Gender	– 0.083	0.440	–
Diabetes duration	0.202	0.060	–
BMI	– 0.191	0.076	–
Systolic blood pressure	0.189	0.097	–
Diastolic blood pressure	– 0.079	0.488	–
Fasting glucose	0.104	0.334	–
$HbA1_c$	0.136	0.203	–
Triglycerides	– 0.081	0.452	–
Total cholesterol	– 0.077	0.471	–
HDL cholesterol	– 0.109	0.310	–
LDL cholesterol	– 0.094	0.383	–

Table 3 Determinants of urinary albumin concentration by multivariate analysis

	Model 1 (F)	*Model 2 (F)*
sTNF-R1	32.1	31.0
sTNF-R2	0.2	0.2
Age	1.9	0.7
Serum creatinine	0.1	0.2
BMI	–	0.1
Systolic blood pressure	–	1.8
R2	0.263	0.280

ity. Microalbuminuria has been shown to be not only an indicator of incipient nephropathy but also an independent risk factor for cardiovascular disease [5]. The mechanisms underlying the evolution of microalbuminuria in diabetic patients are not fully clarified. Genetic factors, insulin resistance, glycemic control, blood pressure, smoking, and lipid abnormalities have been implicated in albuminuria development in diabetic patients [23].

Inflammation seems to be associated with urinary albumin excretion in diabetic patients. Gabazza et al. [24] showed high concentrations of serum fibrinogen in type 2 diabetic patients with albuminuria compared to those without albuminuria. Microalbuminuria has been shown to be associated with fibronectin and sialic acid in type 2 diabetic patients [25,26]. Furthermore, Festa et al. [6] have reported an association of CRP and fibrinogens with urinary albumin excretion in the microalbuminuric range of type 2 diabetic individuals. Stehouwer et al. [7] confirmed that increased urinary albumin excretion, endothelial dysfunction, and chronic inflammation are interrelated processes associated with risk of death in type 2 diabetic patients.

In the present study, we investigated the relationship between albuminuria and TNF-α system after carefully matching the participants for smoking, BMI, blood pressure, glycemic control, and lipid profile. We used serum TNF-α, soluble TNF-R1, and soluble TNF-R2 as the index of TNF-α system activity and found, for the first time, that soluble TNF-R1 was independently associated with albuminuria in type 2 diabetic patients. However, we could not find the relationship between albuminuria and TNF-α. The reason is not known, but may be due to circulating TNF receptor levels remaining elevated for a longer time than TNF-α itself and reflecting the degree of TNF-α activation more accurately than the measurement of TNF-α itself. TNF receptor levels might be considered to be a more valuable factor for monitoring the degree of TNF-α system activity. Thus, the TNF-α system could predispose to the development of microalbuminuria in type 2 diabetic patients. Baud and Ardaillou [27] have shown that TNF-α induces glomerular infiltration by leukocytes. Klein et al. [28] have demonstrated that TNF-α influences the metabolism of glycosaminoglycans, which are components of the vascular endothelium and the glomerular basement membrane and are involved in the etiology of microalbuminuria.

The mechanisms for the increased activity of TNF-α system in type 2 diabetic patients with microalbuminuria are unknown; however, elevated synthesis, reduced catabolism, or both must be present. *In vitro* investigations have shown increased TNF-α messenger RNA expression in glomeruli from diabetic rats [29]. Recent studies have demonstrated that advanced glycation end products binding to specific cell-surface receptor molecules expressed on kidney cells may induce local cytokine and initiate local inflammatory reaction [30]. Angiotensin II, a substance associated with development of renal injury in diabetic patients, has been shown to upregulate TNF-α expression [31].

Interestingly, soluble TNF-R1, but not soluble TNF-R2, was associated with albuminuria in our diabetic patients. The reasons for the discrepancy between the TNF-R1 or TNF-R2 relationship to albuminuria in our diabetic patients are not clear. These two receptors seem to differ in terms of signaling and functional properties [8–10]. Several studies have demonstrated that obese subjects overexpress TNF-alpha and TNF-R2 in adipose tissue and have higher concentrations of serum TNF-R2 levels in relation to lean controls [32,33]. TNF-α can upregulate TNF-R2 expression in humans [34]. In contrast, the majority of biological responses classically attributed to TNF-α such as cytotoxicity and nuclear kappa B activation are mediated by TNF-R1 [35]. Pichler et al. [36] have shown that sTNF-R1 may play an important role in the onset of the acute stage of Graves' disease.

Nevertheless, the present study that TNF-R1, but not TNF-R2, is associated with albuminuria in diabetic patients suggests that TNF-R1 may play a role in the evolution of vascular complications in our non-obese type 2 diabetic patients. This idea is supported by the recent study by Rauchhaus et al. [11] demonstrating that elevated soluble TNF-R1 levels are predictive of cardiovascular mortality in patients with chronic heart failure. Furthermore, Zoppini et al. [12] have reported that TNF-R1 is associated with the progression of microalbuminuria and retinopathy in type 1 diabetic patients.

In summary, although our present study was performed among the limited patients that were well-controlled in terms of BMI, HbA_{1c}, blood pressure, LDL cholesterol, triglycerides, total and HDL cholesterol, serum soluble TNF-R1 seems to be associated with albuminuria in non-obese Japanese type 2 diabetic patients. Further study should be undertaken to clarify whether or not serum soluble TNF-R1 is reflective of early stage of atherosclerosis in non-obese Japanese type 2 diabetic patients.

Acknowledgement

This study is supported in part by Health Sciences Research Grants for Comprehensive Research on Aging and Health, and Research for Measures for Intractable Diseases from the Ministry of Health, Labor and Welfare.

References

[1] Fuller H, Shipley MJ, Rose G, Jarrett RJ, Keen H. Coronary heart disease risk and impaired glucose tolerance. Lancet 1980; 1: 1373 – 1376

[2] Yano K, Kagan A, McGee D, Rhoads GG. Glucose intolerance and nine-year mortality in Japanese men in Hawaii. Am J Med 1982; 72: 71 – 80

[3] Diabetes Drafting Group. Prevalence of small vessel and large vessel disease in diabetic persons from 14 centers: the World Health Organization multinational study of vascular disease in diabetics. Diabetologia 1985; 28: 615 – 640

[4] Herman JB, Medalie JH, Goldbourt U. Differences in cardiovascular morbidity and mortality between previously known and newly diagnosed adult diabetics. Diabetologia 1977; 13: 229 – 234

[5] Mogensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. N Engl J Med 1984; 310: 356 – 360

[6] Festa A, D'Agostino R, Howard G, Mykkanen L, Tracy RP, Haffner SM. Inflammation and microalbuminuria in nondiabetic and type 2 diabetic subjects: The insulin resistance atherosclerosis study. Kidney Int 2000; 58: 1703 – 1710

[7] Stehouwer CDA, Gall MA, Twisk JWR, Knudsen E, Emeis JJ, Parving HH. Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes. Progressive, interrelated, and independently associated with risk of death. Diabetes 2002; 51: 1157 – 1165

[8] Smith CA, Farrah T, Goodwin RG. TNF receptor superfamily of cellular and viral proteins: activation, costimulation and death. Cell 1994; 76: 959 – 962

[9] Tartaglia LA, Weber RF, Figari IS, Reynolds S, Pailadino MA Jr, Goeddel DV. The two different receptors. Proc Natl Acad Sci USA 1991; 88: 9292 – 9296

[10] Tartaglia LA, Goeddel DV. Two TNF receptors. Immunol Today 1992; 13: 151 – 153

[11] Rauchhaus M, Doehner W, Francis DP, Davos C, Kemp M, Liebenthal C, Niebauer J, Hooper J, Volk HD, Coats AJS, Anker SD. Plasma cytokine parameters and mortality in patients with chronic heart failure. Circulation 2000; 102: 3060 – 3067

[12] Zoppini G, Faccini G, Muggeo M, Zenari L, Falezza G, Targher G. Elevated plasma levels of soluble receptors of TNF-α and their association with smoking and microvascular complications in young adults with type 1 diabetes. J Clin Endocrinol Metab 2001; 186: 3805 – 3808

[13] Hotamisligil GS, Shargil NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993; 259: 87 – 91

[14] Tanaka S, Seino H, Satoh J, Fujii N, Rikiishi H, Zhu XP, Takahashi K, Sagara M, Nobunaga T, Toyota T. Increased in vivo production of tumor necrosis factor after development of diabetes in non-treated, long-term diabetic BB rats. Clin Immunol Immunopathol 1992; 62: 258 – 263

[15] Taniguchi A, Nakai Y, Doi K, Fukuzawa H, Fukushima M, Kawamura H, Tokuyama K, Suzuki M, Fujitani J, Tanaka H, Nagata I. Insulin sensitivity, insulin secretion, and glucose effectiveness in obese subjects: a minimal model analysis. Metabolism 1995; 44: 1397 – 1400

[16] World Health Organization. Diabetes Mellitus: Report of a WHO Study Group. Geneva: World Health Org, 1985; (Tech. Rep. Ser., no 727)

[17] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499 – 502

[18] Nakai Y, Hamagaki S, Seino Y, Takagi R, Taniguchi A, Kurimoto F. Plasma concentrations of tumor necrosis factor-α (TNF-α) and soluble TNF receptors in patients with anorexia nervosa. J Clin Endocrinol Metab 1999; 84: 1226 – 1228

[19] Gatling W, Knight C, Hill RD. Screening for early diabetic nephropathy: which sample to detect microalbuminuria? Diabetic Med 1985; 2: 451 – 455

[20] Cowell CT, Rodgers S, Silkink M. First morning urinary albumin concentration is a good predictor of 24-hour urinary albumin excretion in children with type 1 (insulin-dependent) diabetes. Diabetologia 1986; 29: 97 – 99

[21] Hutchison AS, O'Reilly DSJ, McCuish AC. Albumin excretion rate, albumin concentration, and albumin/creatinine ratio compared for screening diabetics for slight albuminuria. Clin Chem 1988; 34: 2019 – 2021

[22] American Diabetes Associations. Nephropathy in diabetes. Diabetes Care 2004; 27: 579 – 583

[23] Groop L, Ekstrand A, Forsblom C, Widen E, Groop PH, Teppo AM, Eriksson J. Insulin resistance, hypertension, and microalbuminuria in patients with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 1993; 36: 642 – 647

[24] Gabazza EC, Takeya H, Debuchi H, Sumida Y, Taguchi O, Murata K, Nakatani K, Yano Y, Mohri M, Sata M, Shima T, Nishioka J, Suzuki K. Protein C activation in NIDDM patients. Diabetologia 1996; 39: 1455 – 1461

[25] Nielsen S, Schmitz A, Bacher T, Rehling M, Ingerslev J, Mogensen CE. Transcapillary escape rate and albuminuria in type II diabetes: Effects of short-term treatment with low-molecular weight heparin. Diabetologia 1999; 42: 60 – 67

[26] Chen JW, Gall MA, Yokoyama H, Jensen JS, Deckert M, Parving HH. Raised serum sialic acid concentration in NIDDM patients with and without diabetic nephropathy. Diabetes Care 1996; 19: 130 – 134

[27] Baud L, Ardaillou R. Tumor necrosis factor alpha in glomerular injury. Kidney Int 1994; 45 (Suppl 45): S32 – S36

[28] Klein NJ, Shennan GI, Heyderman RS, Levin M. Alteration in glycosaminoglycan metabolism and surface charge on human umbilical vein endothelial cells induced by cytokines, endotoxin, and neutrophils. J Cell Sci 1992; 102: 821 – 832

[29] Nakamura T, Fukui M, Ebihara I, Osada S, Nagaoka I, Tomino Y, Koide H. mRNA expression of growth factors in glomeruli from diabetic rats. Diabetes 1993; 42: 450 – 456

[30] Basta G, Lazzerini G, Massaro M, Simoncini T, Tanganelli P, Fu C, Kislinger T, Stern DM, Schmidt AM, De Caterina R. Advanced glycation end products activate endothelium through signal-transduction receptor RAGE. A mechanism for amplification of inflammatory responses. Circulation 2002; 105: 816 – 822

[31] Ruits-Ortega M, Lorenzo O, Suzuki Y, Ruperez M, Egido J. Proinflammatory actions of angiotensins. Curr Opin Nephrol Hypertens 2001; 10: 321 – 329

[32] Hotamisligil GS, Arner P, Atkinson RL, Spiegelman M. Differential regulation of the p80 tumor necrosis factor receptor in human obesity and insulin resistance. Diabetes 1997; 46: 451 – 455

[33] Fernandez-Real JM, Broch M, Ricart W, Casmitjana R, Gutierrez C, Vendrell J, Richart C. Plasma levels of the soluble fraction of tumor necrosis factor 2 and insulin resistance. Diabetes 1998; 47: 1757 – 1762

[34] Liu LS, Spelleken M, Rohrig K, Hauner H, Eckel J. Tumor necrosis factor-α acutely inhibits insulin signaling in human adipocytes. Implication of the p80 tumor necrosis factor receptor. Diabetes 1998; 47: 515 – 522

[35] Vandenabeele P, Declercq W, Beyaert R, Fiers W. Two tumor necrosis factor receptors: structure and function. Trends in Cell Bilogy 1995; 5: 392 – 399

[36] Pichler R, Maschek W, Hatzl-Griesenhofer M, Huber H, Crespillo-Gomez C, Berg J. Soluble tumor necrosis factor-α receptor 1 and interleukin-6 as markers of activity in thyrotoxic Graves' disease. Horm Metab Res 2003; 35: 427 – 433

Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 1297–1301

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Three measures of tumor necrosis factor α activity and insulin resistance in nonobese Japanese type 2 diabetic patients

Michihiro Ohya[a], Ataru Taniguchi[a,*], Mitsuo Fukushima[b], Yoshikatsu Nakai[c], Yukiko Kawasaki[d], Shoichiro Nagasaka[e], Akira Kuroe[a], Yoshiro Taki[a], Satoru Yoshii[a], Masaya Hosokawa[d], Nobuya Inagaki[d], Yutaka Seino[a,d]

[a]*Division of Diabetes and Clinical Nutrition, Kansai-Denryoku Hospital, Osaka 553-0003, Japan*
[b]*Department of Health Informatics Research, Translational Research Informatics Center, Foundation for Biochemical Research and Innovation, Kobe 650-0047, Japan*
[c]*School of Health Sciences Faculty of Medicine, Kyoto University, Kyoto 606-8507, Japan*
[d]*Department of Diabetes and Clinical Nutrition, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan*
[e]*Division of Endocrinology and Metabolism, Jichi Medical School, Tochigi, Japan*

Received 4 December 2004; accepted 5 April 2005

Abstract

The aim of the present study was to investigate the relationship between insulin resistance and tumor necrosis factor α (TNF-α) as well as soluble TNF receptors (sTNF-R), body mass index (BMI), leptin, adiponectin, and serum lipid profile including triglycerides in nonobese Japanese patients with type 2 diabetes. A total of 88 nonobese Japanese type 2 diabetic patients were studied. The duration of diabetes was 11.0 ± 0.8 years. In conjunction with BMI, glycosylated hemoglobin (HbA1c), fasting concentrations of plasma glucose, serum lipids (triglycerides, high-density lipoprotein cholesterol, and total cholesterol), serum leptin, serum adiponectin, serum TNF-α, and soluble TNF receptors (sTNF-R1 and sTNF-R2) were also measured. Insulin resistance was estimated by the insulin resistance index of homeostasis model assessment. Insulin resistance was positively correlated with BMI, triglycerides, leptin, and total cholesterol and negatively correlated with adiponectin and high-density lipoprotein cholesterol. In contrast, insulin resistance was not associated with TNF-α, nor sTNF-R (sTNF-R1 and sTNF-R2) in our diabetic patients. There was no significant relationship between the 3 measures of TNF-α system (TNF-α, sTNF-R1, and sTNF-R2) and BMI, serum triglycerides, leptin, or adiponectin in these patients. From these results, it can be concluded that peripheral levels of TNF-α system activity are not a major factor responsible for insulin resistance in nonobese Japanese type 2 diabetic patients.

1. Introduction

Type 2 diabetes mellitus is a heterogeneous syndrome characterized by insulin resistance and/or defective insulin secretion [1]. In contrast to white populations, nonobese Japanese patients with type 2 diabetes are unique in that they are divided into 2 variants: one with insulin resistance and the other with normal insulin sensitivity [2-9]. The former group is characterized by higher body mass index (BMI), higher triglycerides, higher leptin, and lower adiponectin as compared with the latter group. Whereas serum leptin level is shown to be associated with subcutaneous fat area, serum concentrations of triglycerides and adiponectin are linked to visceral fat areas in nonobese Japanese type 2 diabetic patients [7-9]. Thus, the adipose tissue–linked substances are hypothesized to be associated with insulin resistance in nonobese Japanese type 2 diabetic patients.

Tumor necrosis factor α (TNF-α) is 1 of the most important candidates expressed in human adipocytes [10]. Adipocytes of obese subjects are reported to have higher rates in TNF-α messenger RNA expression and TNF-α protein production as compared with those of nonobese subjects, thus resulting in a greater serum TNF-α concentration in obese subjects [11-13]. The increase in TNF-α messenger RNA levels is positively correlated to the degree

* Corresponding author. Fax: +81 6 6458 6994.
E-mail address: k-58403@kepco.co.jp (A. Taniguchi).

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.04.017