Cigarette Smoking and Blood Insulin, Glucose, and Lipids in Young Japanese Women

Yu Kang, Hiroyuki Imamura,* Reika Masuda, and Yuka Noda

Laboratory of Nutrition and Exercise Physiology, Nakamura Gakuen University Graduate School, 5–7–1 Befu, Jonan-ku, Fukuoka 814–0198, Japan

(Received November 5, 2008; Accepted December 24, 2008; Published online January 7, 2009)

The aim of the present study was to examine the effects of cigarette smoking (CS) on fasting blood insulin, homeostasis model assessment index (HOMA-R index), glucose and lipids in Japanese collegiate women. Twenty-six smokers were individually matched for physical activity scores, age, and body mass index with 26 nonsmokers. Information on smoking, physical activity habits, and diet record were collected. Blood samples were taken and analyzed to evaluate their associations with CS. The results showed significantly higher mean serum insulin, HOMA-R index, and glucose while lower mean high-density lipoprotein two cholesterol (HDL_2-C) in smokers as compared with nonsmokers. The mean nutrient intakes showed no significant differences between smokers and nonsmokers. In conclusion, it appears that CS is associated with insulin resistance, impaired fasting glucose and lower HDL_2-C in young Japanese female smokers. This may partly explain the deleterious effects of smoking on coronary heart disease risk.

Key words —— cigarette smoking, insulin resistance, high-density lipoprotein two cholesterol, glucose

INTRODUCTION

Although the prevalence of smoking among Japanese women is significantly lower than that among Japanese men, the prevalence in young Japanese women has been gradually increasing especially for women aged 20–30 years.[1] Studies have indicated that there is a much larger detrimental impact of cigarette smoking (CS) in women.[2,3] Therefore, greater concern should be placed on female smokers.

Insulin resistance and dyslipidemia have been reported to be associated with coronary heart disease (CHD).[4–6] It has been suggested that smoking leads to dyslipidemia including decrease in serum high-density lipoprotein cholesterol (HDL-C) and increase in triglycerides (TG).[7,8] Studies also suggested that smokers had significantly higher plasma insulin concentrations[9–13] and were more insulin resistant[11–14] than nonsmokers, whereas inconsistent results were also found.[15–18] However, these data are based on studies of middle-aged or older individuals conducted in Western countries. Data on young women are scarce. We therefore examined fasting blood insulin, homeostasis model assessment index (HOMA-R index), glucose and lipids among smokers and nonsmokers in Japanese collegiate women. With the concern that unhealthy behaviors may exert their harmful effects on the biomarkers mentioned above, we measured alcohol consumption and physical activity habits and compared nutrient intakes between smokers and nonsmokers.

MATERIALS AND METHODS

Subjects and Self-administered Questionnaire —— Collegiate women were recruited from the Nakamura Gakuen University in 2006 and consented to the procedure after explanation of the purpose of the study. To be included in the study, they had to meet the following criteria: they were menstruating at normal intervals, ranging from 26–31 days, which fell within the normally accepted range;[19] they drank alcohol less than once a week and even then only had a small amount; they were not on any medication at the time of their participation in the study; and they had never taken birth control pills. Smokers were eligible if they smoked at least for one year. Twenty-six current smokers volunteered, who were individually matched for physical activity scores, age and body mass index (BMI) with 26 nonsmokers who had never smoked. The study protocol was approved by the Ethics Committee of the Nakamura Gakuen University. Informed consent was obtained from each subject.

*To whom correspondence should be addressed: Laboratory of Nutrition and Exercise Physiology, Nakamura Gakuen University Graduate School, 5–7–1 Befu, Jonan-ku, Fukuoka 814–0198, Japan. Tel. & Fax: +81-92-851-5964; E-mail: hiro7ima@nakamura-u.ac.jp

Information on smoking and physical activity habits was obtained via a self-administered questionnaire. Accuracy of the questionnaire was checked through individual interviews. Smokers were asked to indicate how many cigarettes a day and how many years did they smoke. The frequency, duration and mode of physical activity were questioned, and scores 1–5 were given according to Young and Steinhardt.[20]

Measurements —— The body weight and height were measured with the subjects in underwear to the nearest 0.1 kg and 0.1 cm, respectively. BMI was expressed as weight/height^2 (kg/m^2).

Dietary information was collected using a 3-weekday diet record. Each diet was analyzed by means of a computer program. Each food item was coded according to the tables of the Japanese foodstuff composition.[21]

Blood Analysis —— Physical exercise was not allowed 48 hr, and beverages other than water and CS were not allowed 24 hr prior to the blood sampling. Subjects arrived at the laboratory by 08:00. The temperature of the laboratory was set at 25°C. Fasting (12 hr) blood samples were drawn from the antecubital vein after each subject had been seated quietly for at least 20 min. All blood samples were taken between days 7 and 9 of the menstrual cycle when estrogen levels were relatively low.[19] The samples were immediately stored on ice, and kept on ice until centrifuged within 10 min in a refrigerated centrifuge at 4°C. Samples were analyzed within 10 days, and all measurements were duplicated. Glucose, total cholesterol (TC) and triglycerides (TG) were analyzed by enzymatic methods. HDL-C was analyzed by direct assay with selective inhibition method. HDL_2-C and HDL_3-C were analyzed by ultracentrifugation method. Low-density lipoprotein cholesterol (LDL-C) was analyzed by heparin and citrate precipitation method. Details of these methods have been presented elsewhere.[22] Insulin was analyzed by an automated chemiluminescence enzyme immunoassay analyzer, BCS 600 (SRL, Inc., Tokyo, Japan).[23] HOMA-R index was calculated as: fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5.[24] The coefficients of variation for intra-assay and inter-assay were 2.0% and 2.0% for glucose, 0.54% and 2.71% for TC, 4.16% and 7.34% for TG, 0.91% and 1.73% for HDL-C, 7.18% and 8.71% for HDL_2-C, 7.27% and 7.33% for HDL_3-C, 1.37% and 3.70% for LDL-C, and 3.86% and 1.57% for insulin, respectively.

Statistical Analysis —— The SPSS Statistics 10.0 version for Windows (SPSS Inc., Chicago, IL, U.S.A.) was used for statistical analysis. The results were expressed as mean ± S.D. throughout the paper. The statistical analysis was done with standard methods for 2-tailed, unpaired Student's *t* test. To improve skewness of the distributions, triglycerides and insulin were logarithmically transformed for statistical analysis. Since untransformed values gave similar results, only these are presented. *P*-values less than 0.05 were considered statistically significant.

RESULTS

The characteristics of study subjects by smoking status are shown in Table 1. Smokers had significantly higher mean fasting blood insulin, HOMA-R index and glucose while lower mean fasting HDL_2-C as compared to nonsmokers. However, CS did not significantly correlated with these parameters (data not shown).

The mean nutrient intakes showed no significant differences between smokers and nonsmokers (Table 2).

DISCUSSION

Our results were consistent with studies that found smokers have higher fasting serum insulin and HOMA-R index than nonsmokers.[9–14] Studies that have been inconsistent with these may be

Table 1. Characteristics of Study Subjects by Smoking Status

	Smokers (n = 26)	Nonsmokers (n = 26)
Age (y)	20.8 ± 0.65	21.0 ± 0.69
BMI (kg/m^2)	20.9 ± 2.18	20.4 ± 1.36
Waist (cm)	71.4 ± 7.20	70.4 ± 3.70
Insulin (μU/ml)	10.2 ± 7.66*	6.6 ± 2.42
HOMA-R index	2.4 ± 1.8*	1.5 ± 0.6
Glucose (mmol/l)	5.2 ± 0.20**	5.0 ± 0.35
TC (mmol/l)	4.5 ± 0.67	4.8 ± 0.60
TG (mmol/l)	0.9 ± 0.39	0.8 ± 0.44
LDL-C (mmol/l)	2.6 ± 0.58	2.5 ± 0.55
HDL-C (mmol/l)	1.5 ± 0.31	1.7 ± 0.42
HDL_2-C (mmol/l)	1.0 ± 0.29*	1.2 ± 0.40
HDL_3-C (mmol/l)	0.5 ± 0.07	0.5 ± 0.06

Data are mean ± S.D. *$p < 0.05$, **$p < 0.01$.

Table 2. Various Nutrient Intakes between Smokers and Nonsmokers According to the Three-weekday Diet Record

	Smokers (n = 26)	Nonsmokers (n = 26)
Energy (kcal)	1584.6 ± 500.0	1582.1 ± 296.3
Protein (g)	54.5 ± 18.6	52.7 ± 10.0
Fat (g)	55.5 ± 24.1	54.5 ± 14.8
Saturated fat (g)	29.6 ± 23.5	22.7 ± 9.1
Carbohydrate (g)	204.6 ± 63.3	214.7 ± 38.8
Potassium (mg)	1891.5 ± 819.7	1829.3 ± 517.7
Calcium (mg)	419.6 ± 214.9	404.2 ± 134.3
Magnesium (mg)	188.3 ± 82.2	196.6 ± 43.4
Phosphorus (mg)	816.8 ± 293.8	795.3 ± 163.6
Iron (mg)	5.9 ± 2.6	6.4 ± 1.6
Zinc (mg)	6.4 ± 2.7	6.5 ± 1.4
Copper (mg)	0.8 ± 0.3	0.9 ± 0.2
Vitamin A (μgRE)	465.8 ± 244.5	672.3 ± 443.5
Vitamin D (μg)	5.6 ± 6.2	3.9 ± 2.5
Vitamin E (mg)	6.5 ± 2.5	7.2 ± 2.2
Vitamin K (μg)	139.3 ± 107.9	162.1 ± 98.6
Vitamin B_1(mg)	0.7 ± 0.4	0.7 ± 0.2
Vitamin B_2(mg)	1.1 ± 0.4	1.0 ± 0.3
Vitamin C (mg)	65.7 ± 46.1	69.4 ± 37.8
Fiber (g)	8.1 ± 3.7	10.6 ± 5.3
Salt (g)	7.5 ± 3.2	6.7 ± 1.9

Data are mean ± S.D. $^{*}p < 0.05$, $^{**}p < 0.01$.

related to the differences in study design, subjects, methods for assessment of insulin resistance and the extent of controlling for confounders.[15–18] Although we lack the power to use statistical techniques for adjustment due to the small sample size, a recent study[25] with a relatively large sample conducted in healthy young subjects showed similar results as ours. Besides, all subjects in the present study were matched for age, BMI, and physical activity scores, which were considered to differ between smokers and nonsmokers.[26–31] It has also been reported that smokers are more likely to have unhealthy dietary practices,[32] however, mean nutrient intakes showed no apparent differences between smokers and nonsmokers in our study. To avoid the influence of acute effects of CS[33] and menstrual cycle phase on insulin and other metabolic factors, the smokers were refrained from smoking for 24 hr before blood sampling, which were taken between days 7 and 9 of the menstrual cycle. Although we took fasting insulin and used indirect method of HOMA-R index to determine insulin resistance, their accuracies have been demonstrated to have substantial correlation with the gold standard method.[34–36]

Investigators have suggested that CS may exert a direct toxic on pancreatic tissue.[37,38] However, our results may not favor this explanation since all subjects were non-diabetic without significant disturbance in insulin secretion. Thus it seems reasonable to speculate that insulin resistance could be caused by the direct effects of nicotine, carbon monoxide, or other agents in tobacco smoke.[11] This assumption was supported by another two research that demonstrated that nicotine may directly lead to insulin resistance.[39,40] Further research is still needed to address the underlying mechanisms.

Studies have reported that CS may alter the glucose metabolism[41,42] and cause higher fasting glucose in smokers.[43,44] Since our two groups did not differ from each other with the major factors that may influence fasting glucose, the observed higher fasting glucose in smokers in our results may be caused by the smoke. However, the responses to stress following the 24 hr of smoking cessation cannot be ruled out.

The association of lower HDL_2-C and CS found in the present study may be secondary to the insulin-increasing effect of smoking[13,45,46] and it has been suggested that HDL_2-C might be a better predictor of CHD than total HDL-C.[47]

In conclusion, compared with nonsmokers, smokers have higher fasting serum glucose, insulin, HOMA-R index, and lower HDL_2-C. Although we matched for age, BMI, and physical activity scores, residual confounding could not be ruled out and might have biased our results. In addition, due to the fact that there might be a close association between smoking and alcohol consumption,[48,49] our recruiting criteria might have excluded certain smokers, particularly heavy smokers, which may also have influenced our results. Furthermore, the small sample size might have restricted our power to find the differences between smokers and nonsmokers with respect to nutrient intakes and thus we could not neglect the role of unhealthy diet when interpreting our findings. Another limitation is that we relied only on self report without using more objective markers such as cotinine to verify smoking status. Therefore, the findings may not be generalized and need to be confirmed by future research in a larger sample size and in other ethnic populations. The reasons why CS increases risk of CHD are unclear. However, we suggested that insulin resistance and lower HDL_2-C in smokers may contribute to their increased risk of CHD.

REFERENCES

1) Ministry of Health, Labor, and Welfare (2003) *Tobacco or Health: the National Nutrition Examination Survey*, Statistic Association, Tokyo (in Japanese).
2) Njolstad, I., Arnesen, E. and Lund-Larsen, P. G. (1996) Smoking, serum lipids, blood pressure, and sex differences in myocardial infarction. A 12-year follow-up of the Finnmark Study. *Circulation*, **93**, 450–456.
3) Prescott, E., Osler, M., Andersen, P. K., Hein, H. O., Borch-Johnsen, K., Lange, P., Schnohr, P. and Vestbo, J. (1998) Mortality in women and men in relation to smoking. *Int. J. Epidemiol.*, **27**, 27–32.
4) Carlson, L. A., Bottiger, L. E. and Ahfeldt, P. E. (1979) Risk factors for myocardial infarction in the Stockholm prospective study: a 14-year follow-up focusing on the role of plasma triglycerides and cholesterol. *Acta Med. Scand.*, **206**, 351–360.
5) Austin, M. A. (1991) Plasma triglyceride and coronary heart disease. *Arterioscler. Thromb.*, **11**, 2–14.
6) Pyörälä, K. (1979) Relationship of glucose tolerance and plasma insulin to the incidence of coronary heart disease: results from two population studies in Finland. *Diabetes Care*, **2**, 131–141.
7) Cullen, P., Schulte, H. and Assmann, G. (1998) Smoking, lipoproteins and coronary heart disease risk. Data from the Munster Heart Study (PROCAM). *Eur. Heart J.*, **19**, 1632–1641.
8) Mammas, I. N., Bertsias, G. K., Linardakis, M., Tzanakis, N. E., Labadarios, D. N. and Kafatos, A. G. (2003) Cigarette smoking, alcohol consumption, and serum lipid profile among medical students in Greece. *Eur. J. Public Health*, **13**, 278–282.
9) Hautanen, A. and Adlercreutz, H. (1993) Hyperinsulinaemia, dyslipidaemia and exaggerated adrenal androgen response to adrenocorticotropin in male smokers. *Diabetologia*, **36**, 1275–1281.
10) Rönnemaa, T. R., Pyörälä, K., Rönnemaa, E. M., Laakso, M. and Puukka, P. (1996) Smoking is independently associated with high plasma insulin levels in nondiabetic men. *Diabetes Care*, **19**, 1229–1232.
11) Facchini, F. S., Hollenbeck, C., Jeppesen, J., Chen, Y.-D. I. and Reaven, G. M. (1992) Insulin resistance and cigarette smoking. *Lancet*, **339**, 1128–1130.
12) Kong, C., Nimmo, L., Elatrozy, T., Anyaoku, V., Hughes, C., Robinson, S., Richmond, W. and Elkeles, R. S. (2001) Smoking is associated with increased hepatic lipase activity, insulin resistance, dyslipidaemia and early atherosclerosis in Type 2 diabetes. *Atherosclerosis*, **156**, 373–378.
13) Targher, G., Alberiche, M., Zenere, M. B., Bonadonna, R. C., Muggeo, M. and Bonora, E. (1997) Cigarette smoking and insulin resistance in patients with noninsulin-dependent diabetes mellitus. *J. Clin. Endocrinol. Metab.*, **82**, 3619–3624.
14) Eliasson, B., Mero, N., Taskinen, M.-R. and Smith, U. (1997) The insulin resistance syndrome and postprandial lipid intolerance in smokers. *Atherosclerosis*, **129**, 79–88.
15) Henkin, L., Zaccaro, D., Haffner, S., Karter, A., Rewers, M., Sholinsky, P. and Waqenknecht, L. (1999) Cigarette smoking, environmental tobacco smoke exposure and insulin sensitivity: the insulin resistance atherosclerosis study. *Ann. Epidemiol.*, **9**, 290–296.
16) Hughes, K., Choo, M., Kuperan, P., Ong, C.-N. and Aw, T.-C. (1998) Cardiovascular risk factors in relation to cigarette smoking: a population-based survey among Asians in Singapore. *Atherosclerosis*, **137**, 253–258.
17) Wareham, N. J., Ness, E. M., Byrne, C. D., Cox, B. D., Day, N. E. and Hales, C. N. (1996) Cigarette smoking is not associated with hyperinsulinemia: evidence against a causal relationship between smoking and insulin resistance. *Metabolism*, **45**, 1551–1556.
18) Janzon, L., Berntorp, K., Hanson, M., Lindell, S.-E. and Trell, E. (1983) Glucose tolerance and smoking: a population study of oral and intravenous glucose tolerance tests in middle-aged men. *Diabetologia*, **25**, 86–88.
19) Lyons Wall, P. M., Choudhury, N., Gerbrandy, E. A. and Truswell, A. S. (1994) Increase of high-density lipoprotein cholesterol at ovulation in healthy women. *Atherosclerosis*, **105**, 71–78.
20) Young, D. R. and Steinhardt, M. A. (1993) The importance of physical fitness versus physical activity for coronary artery disease risk factors: a cross-sectional analysis. *Res. Q. Exerc. Sport*, **64**, 377–384.
21) The Resources Council of the Science and Technology Agency (1987) *The 4th Revised Edition of Tables of Japanese Foodstuff Composition*, Ishiyaku Press, Tokyo (in Japanese).
22) Imamura, H., Katagiri, S., Uchida, K., Miyamoto, N., Nakano, H. and Shirota, T. (2000) Acute effects of moderate exercise on serum lipids, lipoproteins, and apolipoproteins in sedentary young women. *Clin. Exp. Pharmacol. Physiol.*, **27**, 975–979.
23) Hara, Y., Sakamoto, T., Yamada, E., Watanabe, S. and Kobayashi, S. (2000) Fundamental and clinical evaluation of insulin measurement using automatic chemiluminescence enzyme immunoassay analyzer,

BCS600. *J. Med.*, **44**, 939–946.
24) Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F. and Turner, R. C. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, **28**, 987–992.
25) Naya, T., Hosomi, N., Ohyama, H., Ichihara, S., Ban, C. R., Takahashi, T., Taminato, T., Feng, A., Kohno, M. and Koziol, J. A. (2007) Smoking, fasting serum insulin, and obesity are the predictors of cartotid atherosclerosis in relatively young subjects. *Angiology*, **58**, 677–684.
26) Raitakari, O. T., Porkka, K. V. K., Räsänen, L. and Viikari, J. S. A. (1994) Relations of life-style with l lipids, blood pressure and insulin in adolescents and young adults: the cardiovascular risk in young Finns study. *Atherosclerosis*, **111**, 237–246.
27) Imamura, H., Tanaka, K., Hirae, C., Futagami, T., Yoshimura, Y., Uchida, K., Tanaka, A. and Kobata, D. (1996) Relationship of cigarette smoking to blood pressure and serum lipids and lipoproteins in men. *Clin. Exp. Pharmacol. Physiol.*, **23**, 397–402.
28) Wood, P. D. and Haskell, W. L. (1979) The effect of exercise on plasma high density lipoproteins. *Lipids*, **14**, 417–427.
29) Suter, E. and Hawes, M. R. (1993) Relationship of physical activity, body fat, diet, and blood lipid profile in youths. *Med. Sci. Sports Exerc.*, **25**, 748–754.
30) Erikssen, J. and Enger, S. C. (1978) The effect of smoking on selected coronary heart disease risk factors in middle-aged men. *Acta Med. Scand.*, **203**, 27–30.
31) Ortlepp, J. R., Metrikat, J., Albrecht, M., Maya-Pelzer, P., Pongratz, H. and Hoffmann, R. (2003) Relation of body mass index, physical fitness, and the cardiovascular risk profile in 3127 young normal weight men with an apparently optimal lifestyle. *Int. J. Obes.*, **27**, 979–982.
32) Dallongeville, J., Marecaux, N., Fruchart, J.-C. and Amouye, P. (1998) Cigarette smoking is associated with unhealthy patterns of nutrient intake: a meta-analysis. *J. Nutr.*, **128**, 1450–1457.
33) Swank, A. M. and Fell, R. D. (1990) Effects of acute smoking and exercise on high-density lipoprotein-cholesterol and subfractions in black female smokers. *Metabolism*, **39**, 343–348.
34) Malita, F. M., Karelis, A. D., St-Pierre, D. H., Garrel, D., Bastard, J. P., Tardif, A., Prud'homme, D. and Rabasa-Lhoret, R. (2006) Surrogate indexes vs. euglycaemic-hyperinsulinemic clamp as an indicatior of insulin resistance and cardiovascular risk factors in overweight and obese postmenopausal women. *Diabetes Metab.*, **32**, 251–255.
35) Laakso, M. (1993) How good a marker is insulin level for insulin resistance? *Am. J. Epidemiol.*, **137**, 959–965.
36) Muniyappa, R., Lee, S., Chen, H. and Quon, M. J. (2008) Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am. J. Physiol. Endocrinol. Metab.*, **294**, E15–E26.
37) Rimm, E. B., Manson, J. E., Stampfer, M. J., Colditz, G. A., Willett, W. C., Rosner, B., Hennekens, C. H. and Speizer, F. E. (1993) Cigarette smoking and the risk of diabetes in women. *Am. J. Public Health*, **83**, 211–214.
38) Rimm, E. B., Chan, J., Stampfer, M. J., Colditz, G. A. and Willett, W. C. (1995) Prospective study of cigarette smoking, alcohol use, and the risk of diabetes in men. *BMJ*, **310**, 555–559.
39) Eliasson, B., Taskinen, M. R. and Smith, U. (1996) Long-term use of nicotine gum is associated with hyperinsulinemia and insulin resistance. *Circulation*, **94**, 878–881.
40) Assali, A. R., Beigel, Y., Schreibman, R., Shafer, Z. and Fainaru, M. (1999) Weight gain and insulin resistance during nicotine replacement therapy. *Clin. Cardiol.*, **22**, 357–360.
41) Cryer, P. E., Haymond, M. W., Santiago, J. V. and Shah, S. D. (1976) Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. *N. Engl. J. Med.*, **295**, 573–577.
42) Sandberg, H., Roman, L., Zavodnick, J. and Kupers, N. (1973) The effect of smoking on serum somatotropin, immunoreactive insulin and blood glucose levels of young adult males. *J. Pharmacol. Exp. Ther.*, **184**, 787–791.
43) Houston, T. K., Person, S. D., Pletcher, M. J., Liu, K., Iribarren, C. and Kiefe, C. I. (2006) Active and passive smoking and development of glucose intolerance among young adults in a prospective cohort: CARDIA study. *BMJ*, **332**, 1064–1069.
44) Nakanishi, N., Nakamura, K., Matsuo, Y., Suzuki, K. and Tatara, K. (2000) Cigarette smoking and risk for impaired fasting glucose and type 2 diabetes in middle-aged Japanese men. *Ann. Intern. Med.*, **133**, 183–191.
45) Reaven, G. M. (1998) Role of insulin resistance in human disease. *Diabetes*, **37**, 1595–1607.
46) Manolio, T. A., Savage, P. J., Burke, G. L. Liu, K. A., Wagenknecht, L. E., Sidney, S., Jacobs, D. R., Jr., Roseman, J. M., Donahue, R. P. and Oberman, A. (1990) Association of fasting in-

sulin with blood pressure and lipids in young adults: the Cardia study. *Arteriosclerosis*, **10**, 430–436.

47) Bakogianni, M. C., Kalofoutis, C. A., Skenderi, K. I. and Kalofoutis, A. T. (2001) Clinical evaluation of plasma high-density lipoprotein subfractions (HDL_2, HDL_3) in non-insulin-dependent diabetes with coronary artery disease. *J. Diabetes Complications*, **15**, 265–269.

48) Imamura, H., Uchida, K. and Kobata, D. (2000) Relationship of cigarette smoking with blood pressure, serum lipids and lipoproteins in young Japanese women. *Clin. Exp. Pharmacol. Physiol.*, **27**, 364–369.

49) Gaziano, J. M., Buring, J. E., Breslow, J. L., Goldhaber, S. Z., Rosner, B., VanDenburgh, M., Willett, W. and Hennekens, C. H. (1993) Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. *N. Engl. J. Med.*, **329**, 829–834.