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BRIEF REPORT

Effects of Diet-Induced Moderate Weight Reduction on Intrahepatic and Intramyocellular Triglycerides and Glucose Metabolism in Obese Subjects

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Context: Although moderate weight reduction is recommended as primary therapy of metabolic syndrome, little information is known regarding metabolic changes associated with moderate weight reduction in nondiabetic obese subjects.

Objective: The aim of this study was to determine the effects of a moderate weight reduction program on intracellular lipid and glucose metabolism in muscle and liver.

Participants: Data for 13 nondiabetic obese subjects were evaluated.

Intervention: Subjects were put on a 3-month mildly hypocaloric diet therapy (~35 kcal/kg of ideal body weight).

Main Outcome Measures: Intrahepatic lipid (IHL) and intramyocellular lipid were measured by using ^{1}H magnetic resonance spectroscopy. Peripheral insulin sensitivity and splanchnic glucose uptake were evaluated by euglycemic-hyperinsulinemic clamp with oral glucose load.

Results: Diet therapy for 3 months resulted in 6% reduction in body weight (from 99.9 ± 7.3 to 93.8 ± 6.6 kg, $P < 0.0001$). This change was accompanied by reduction of plasma glucose and insulin excursions during 75-g oral glucose tolerance tests, decrease in diastolic blood pressure, glycated hemoglobin, serum low-density lipoprotein cholesterol, and triglyceride. These changes were also accompanied by a decrease in IHL (from 12.9 to 8.2%, $P < 0.01$) and increase in splanchnic glucose uptake (from 13.5 to 35.0%, $P < 0.03$). On the other hand, the diet program did not affect intramyocellular lipid or glucose infusion rate during euglycemic hyperinsulinemic clamp.

Conclusions: Our results suggest that moderate weight reduction in obese subjects decreased IHL and augmented splanchnic glucose uptake. This mechanism is at least in part involved in improvement of glucose metabolism by moderate weight reduction in obese subjects. (***J Clin Endocrinol Metab* 92: 3326–3329, 2007**)

THE INTERNATIONAL DIABETES FEDERATION recently recommended moderate weight loss (5–10%) by lifestyle modification as a primary therapy of metabolic syndrome (1). It is widely known that moderate weight reduction improves the risk factors of cardiovascular disease (2). Importantly, moderate weight loss also improves insulin resistance and central obesity, which is located upstream of the metabolic syndrome (3).

Recent data suggest that intracellular lipids in muscle and liver are associated with insulin resistance. Cross-sectional studies using ^{1}H-magnetic resonance spectroscopy (^{1}H-MRS) or biopsy specimen have demonstrated that the intramyocellular lipid (IMCL) is associated with insulin resistance in skeletal muscle (4, 5). Hepatic insulin sensitivity was negatively correlated with the IHL content (6, 7). We found that 2 wk of diet with exercise decreased IMCL and increased muscle insulin-mediated glucose uptake, whereas diet with or without exercise decreased IHL by about 25% in type 2 diabetes (8). In addition, our preliminary data suggested that splanchnic glucose uptake (SGU) was improved concomitant with decreased IHL (8). Furthermore, Petersen *et al.* (9) also demonstrated that moderate weight reduction by moderate hypocaloric very-low-fat diet (3%) results in a decrease in IHL and amelioration of hepatic insulin resistance in type 2 diabetes. These data suggest that insulin resistance may be caused by intracellular lipid accumulation in the insulin target organs, and lifestyle modification may reverse these factors.

The present study was designed to determine the effect of moderate weight reduction on intracellular lipid, peripheral insulin resistance, and SGU in nondiabetic obese subjects. In this study, moderate body weight loss was achieved by calorie restriction for 3 months.

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Abbreviations: EGP, Endogenous glucose production; FFA, free fatty acid; GIR, glucose infusion rate; ^{1}H-MRS, ^{1}H-magnetic resonance spectroscopy; IHL, intrahepatic lipid; IMCL, intramyocellular lipid; OGL, oral glucose load; OGTT, oral glucose tolerance test; SGU, splanchnic glucose uptake.

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Subjects and Methods

Experimental subjects and study design

The study subjects were 17 nondiabetic, weight-stable obese men with body mass index (BMI) of more than 30 kg/m^2. All subjects gave written informed consent to the study, which was approved by the Ethics Committee of Juntendo University. No subjects were taking any medications known to affect glucose metabolism.

Subjects were fasted overnight from 2100 h to 0800 h before each baseline measurement. Seventy-five-gram oral glucose tolerance test (OGTT) was performed, and a fasting blood sample was collected for other biochemical tests. Total body fat content was measured by the gas dilution method using a specific analyzer (Bod Pod; Life Measurement Inc., Concord, CA). IMCL in the right tibialis anterior muscle and IHL of segment 6 of the liver were measured by ^{1}H-MRS as previously described (8). In addition, intraabdominal and sc fat in a region extending from 8 cm above to 8 cm below the fourth and fifth lumber interspace (16 slices of slice thickness 10 mm) were measured by magnetic resonance imaging (VISART EX V4.40; Toshiba, Tokyo, Japan) as previously described (10) (n = 8). On a separate day, peripheral insulin sensitivity and SGU were evaluated by the euglycemic hyperinsulinemic clamp using the oral glucose load (OGL) method as previously described (11) (n = 8). Briefly, with the use of artificial endocrine pancreas (STG22; Nikkiso, Shizuoka, Japan), euglycemic-hyperinsulinemic clamp (target plasma glucose of 95 mg/dl and insulin infusion rate of 100 mU/m^2·min) was applied, and the mean glucose infusion rate (GIR) from 105–120 min after insulin infusion was used as a marker of peripheral insulin sensitivity. Then, glucose was administered orally at a dose of 0.5 g/kg body weight, and GIR was diminished to maintain a euglycemic condition for 180 min. SGU was calculated as described previously (11). Briefly, total amount of SGU was calculated from the difference between the amount of ingested glucose and the summation of GIR decrements after glucose ingestion. Then, SGU was expressed as the percentage of ingested glucose amount. The accuracy of SGU measurement has been well discussed in previous reports (11–14).

In addition, a well-trained dietician calculated total energy intake and diet composition from 3-d food diaries. Mean physical activity level for 3 months was estimated with an ambulatory accelerometer (Lifecorder; Suzuken, Nagoya, Japan) (8). Then, all subjects were instructed to consume a nutritionally balanced diet (35 kcal/kg ideal body weight) that consisted of about 25% of energy as fat, about 60% as carbohydrate, and about 15% as protein by the dietitian. All subjects used formula diet Obecure (USCure Inc., Tokyo, Japan) once a day, as an adjunct to the nutritionally balanced diet. All subjects were instructed to visit our hospital every week and were encouraged to maintain calorie restriction by the dietician.

Even after completion of the 3-month calorie restriction period, four subjects failed to achieve 5–10% body weight reduction. Thus, in this study, we excluded these four subjects from this study. In the remaining 13 patients, we reevaluated all the parameters described above after the 3-month calorie restriction period.

Statistical analysis

All data are expressed as the mean ± SD. Baseline data were compared with those obtained after treatment by the paired *t* test. Simple linear regression analysis was performed to evaluate metabolic parameters. Statistical significance was set at $P < 0.05$.

Results

Metabolic parameters

Table 1 shows the clinical characteristics of 13 study subjects before and 3 months after the treatment. The weight loss regimen involved 522 kcal/d calorie reduction during the 3-month period. Although protein intake did not change during the period, carbohydrate (from 1456 ± 296 to 1156 ± 202 kcal, $P < 0.01$) and fat consumption (from 860 ± 144 to 637 ± 137 kcal, $P < 0.01$) was significantly decreased. On the other hand, no significant change in daily physical activity was observed. Thus, the moderate weight reduction observed in our subjects was mainly achieved by calorie restriction of carbohydrate and fat. The weight loss regimen resulted in a significant but only 6% fall in body weight in 3 months. Although the mean BMI value was still above 30 kg/m^2 and the mean waist circumstance was also markedly higher than the limit of metabolic syndrome (85 cm) for Japanese men, diastolic blood pressure decreased significantly during the period. In addition, the weight loss regimen resulted in significant improvement of fasting plasma insulin, HOMA-IR, glycated hemoglobin, serum low-density lipoprotein-cholesterol, and triglyceride but no significant increase in serum adiponectin. Both the areas under the curve of plasma glucose and insulin during OGTT were significantly decreased after the weight reduction program (Table 1).

TABLE 1. Clinical parameters at baseline and after 3-month calorie restriction program

	Baseline	End of study
Calorie intake (kcal/d)	2713 ± 344	2192 ± 305[a]
Physical activity (kcal/d)	428 ± 85	450 ± 51
Body weight (kg)	99.9 ± 7.3	93.8 ± 6.6[a]
Body mass index (kg/m^2)	32.5 ± 2.2	30.5 ± 1.6[a]
Waist (cm)	105 ± 5	101 ± 4[a]
Systolic blood pressure (mm Hg)	124 ± 14	122 ± 13
Diastolic blood pressure (mm Hg)	87 ± 9	80 ± 8[b]
Fasting plasma glucose (mg/dl)	98.4 ± 10.2	97.5 ± 11.2
Fasting plasma insulin (μU/ml)	20.3 ± 7.4	14.0 ± 5.5[a]
HOMA-IR [(mg/dl) × (μU/ml)/405]	4.9 ± 1.8	3.3 ± 1.3[a]
Glycated hemoglobin (%)	5.0 ± 0.3	4.8 ± 0.2[a]
AUC-PG during OGTT	465 ± 74	440 ± 84[c]
AUC-Is during OGTT	290 ± 98	225 ± 74[b]
HDL cholesterol (mg/dl)	51 ± 9	48 ± 7
LDL cholesterol (mg/dl)	145 ± 28	129 ± 22[a]
Triglyceride (mg/dl)	170 ± 78	134 ± 56[b]
FFA (mmol/liter)	0.57 ± 0.14	0.52 ± 0.22
Leptin (ng/ml)	10.6 ± 3.6	8.1 ± 3.0[a]
Adiponectin (μg/ml)	4.1 ± 1.7	4.7 ± 1.5
Total body fat (kg)	31.8 ± 4.9	28.3 ± 5.3[a]
Visceral fat (cm^3)	1819 ± 203	1473 ± 127[a]
Subcutaneous fat (cm^3)	2822 ± 557	2319 ± 393[b]

Data are mean ± SEM of 13 subjects, except visceral fat and sc fat (n = 8). AUC-Is, Area under the curve of insulin; AUC-PG, area under the curve of plasma glucose; HOMA-IR, homeostasis model assessment for insulin resistance.

[a] $P < 0.01$; [b] $P < 0.03$; [c] $P < 0.05$ *vs.* baseline.

Intracellular fat accumulation and glucose metabolism in muscle and liver

The steady-state insulin levels were comparable between the pre- and posttreatment period at 120 min (pre, 266.6 ± 75.5 μU/ml; post, 250.1 ± 35.4 μU/ml) and 300 min (pre, 241.7 ± 86.5 μU/ml; post, 272.9 ± 50.3 μU/ml) during the clamp study. The steady-state GIR before OGL, mainly reflecting glucose uptake in skeletal muscle, was not significantly improved by the diet therapy (from 5.63 ± 1.06 to 6.39 ± 1.23 mg/kg·min, $P = 0.22$; Fig. 1A). SGU was significantly increased from 13.5 ± 20.0 to 35.0 ± 30.8% ($P = 0.023$; Fig. 1B). This metabolic change in the liver was associated with a 36% reduction of IHL (from 12.9 ± 6.2 to 8.2 ± 4.6%, $P = 0.023$; Fig. 1D). On the other hand, no significant change was observed in IMCL (from 2.95 ± 1.29 to 3.11 ± 1.71, $P = 0.70$), similar to GIR (Fig. 1C).

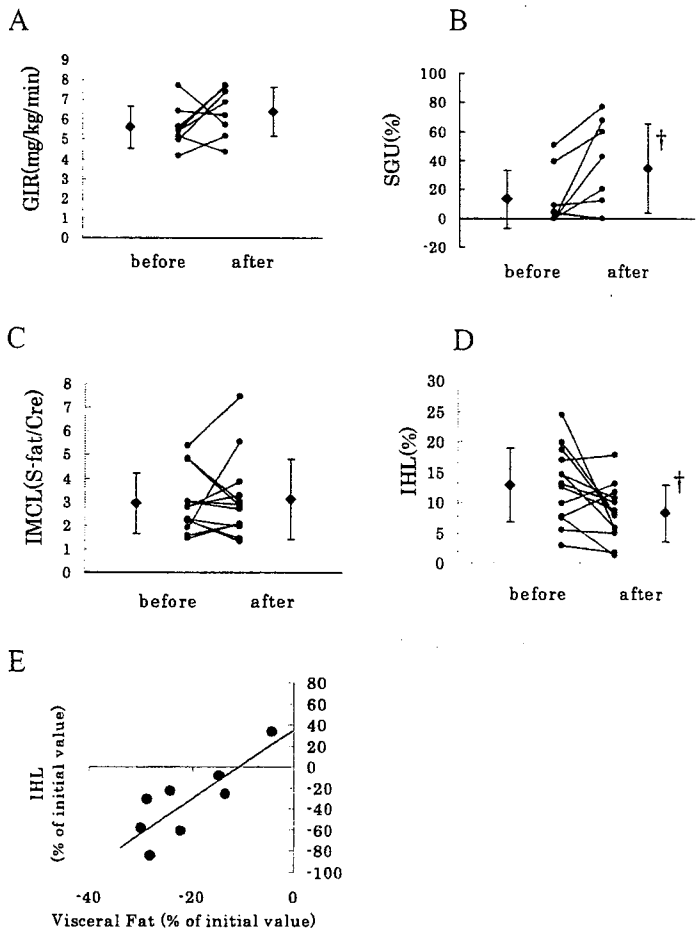

FIG. 1. The changes of glucose uptake and intracellular lipids in muscle and liver by caloric restriction. A and B, GIR (A) and SGU were evaluated by euglycemic-hyperinsulinemic clamp with OGL before and after calorie restriction program (n = 8); C and D, IMCL (C) and IHL (D) were measured by ^{1}H-MRS before and after calorie restriction program. IMCL was quantified by methylene signal intensity (S-fat) at approximately 1.25 ppm and the creatine signal at 3.0 ppm (Cre) as the reference, and calculated as a ratio relative to Cre (S-fat/Cre). IHL was quantified by S-fat and H_2O at about 4.7 ppm as the internal reference and calculated as a percentage of H_2O + S-fat [S-fat × 100/(H_2O + S-fat)] (n = 13). E, Correlation between rate of reduction of IHL and that of visceral fat volume. Changes in IHL correlated significantly with the percent change in visceral fat content (r = 0.823; $P < 0.01$; n = 8).

Total fat and its distribution

As shown in Table 1, total body fat, visceral fat, and abdominal sc fat each significantly decreased after the weight reduction program. Although the rate of reduction of IHL did not correlate with that of total fat mass (r = 0.334; P = 0.27) or abdominal sc fat (r = 0.519; P = 0.20), it correlated significantly with the rate of reduction of visceral fat (r = 0.823; $P < 0.01$) (Fig. 1E).

Discussion

The present study evaluated the effects of moderate weight loss by calorie restriction for 3 months in nondiabetic obese male subjects. The moderate weight reduction resulted in reduction of body fat and IHL but not IMCL. This change coincided with an increase in SGU but not in peripheral insulin sensitivity. In addition, the reduction in IHL correlated closely with reduction in visceral fat but not sc fat.

Recently, Petersen *et al.* (9) showed that 8% weight reduction by a moderately hypocaloric very-low-fat diet within 7 wk results in 80% reduction of IHL in type 2 diabetes, and this reduction was associated with improvement in hepatic insulin resistance. In the present study, moderate weight loss resulted in reduction of IHL, which was associated with an increase in SGU. Our data, together with those of others (9), demonstrate that moderate weight loss by calorie restriction can improve glucose metabolism in the liver with reduction of hepatic fat accumulation.

We reported previously that a 2-wk diet plus exercise therapy in hospitalized type 2 diabetes patients resulted in 19% reduction of IMCL and 56% increase in peripheral insulin sensitivity (8). These changes were not observed in patients on diet restriction but no exercise therapy (8). With regard to these findings, Petersen *et al.* (9) did not observe any changes in IMCL and peripheral insulin sensitivity by a 7-wk program of a moderately hypocaloric very-low-fat diet (3%). Furthermore, in the present study, no significant changes in physical activity, IMCL, and GIR were observed. Thus, increased physical activity by exercise therapy seems to be needed to reduce IMCL and improve insulin resistance in muscle.

In the present study, restriction of fat intake was estimated as approximately 220 kcal/d. A previous study revealed that about 15% of liver fat content was accounted for by dietary fat in nonalcoholic fatty liver patients (15). In addition, several studies showed that dietary fat intake was closely associated with IHL (8) (10, 16). On the other hand, several studies described a link among visceral fat mass, hepatic free fatty acid (FFA) load, and hepatic fat accumulation (16–18). The present study also showed a significant correlation between the percent reduction in visceral fat volume and that

in IHL. Although we did not directly measure the FFA kinetics, this result suggests that reduction of hepatic FFA load originating from dietary fat and visceral fat may be involved in the reduction of IHL seen in the present study.

With the decrease in IHL by moderate weight loss, SGU, which at least in part reflects hepatic glucose uptake (12), was significantly increased in the present study. Similarly, our previous study showed improvement in SGU after 2 wk of diet therapy with or without exercise therapy, which was associated with IHL reduction (8). Bajaj *et al.* (14) also demonstrated that pioglitazone treatment decreased IHL and increased SGU. These results suggest the presence of a tight link between reductions in IHL and rises in SGU. Impairment of SGU could be due to one or more of the following mechanisms: poor glycemic control (13, 14) and lower glucokinase activity (19). However, the exact mechanism that explains the link between IHL and SGU remains unclear.

In this study, estimation of SGU is based on the assumption that endogenous glucose production (EGP) is suppressed during the clamp study and not changed after OGL. However, we cannot exclude the possibility that EGP after OGL might be affected by dietary intervention. Regarding these concerns, EGP level should be theoretically suppressed under a hyperinsulinemic state. In addition, previous reports described that glucagon level was decreased during euglycemic-hyperinsulinemic clamp and not changed after OGL in any metabolic states (12, 20). Although we should take the study limitation into consideration, as previously described, we can reasonably estimate SGU using this method.

Recently, the importance of metabolic syndrome as a risk factor for the onset of cardiovascular disease and progression of type 2 diabetes has been emphasized (1). Our data suggest that moderate weight loss (5–10%) resulted in reduction of IHL, which in turn resulted in improvement of SGU. This metabolic change in the liver might explain the beneficial effect of moderate weight reduction in the prevention of cardiovascular disease.

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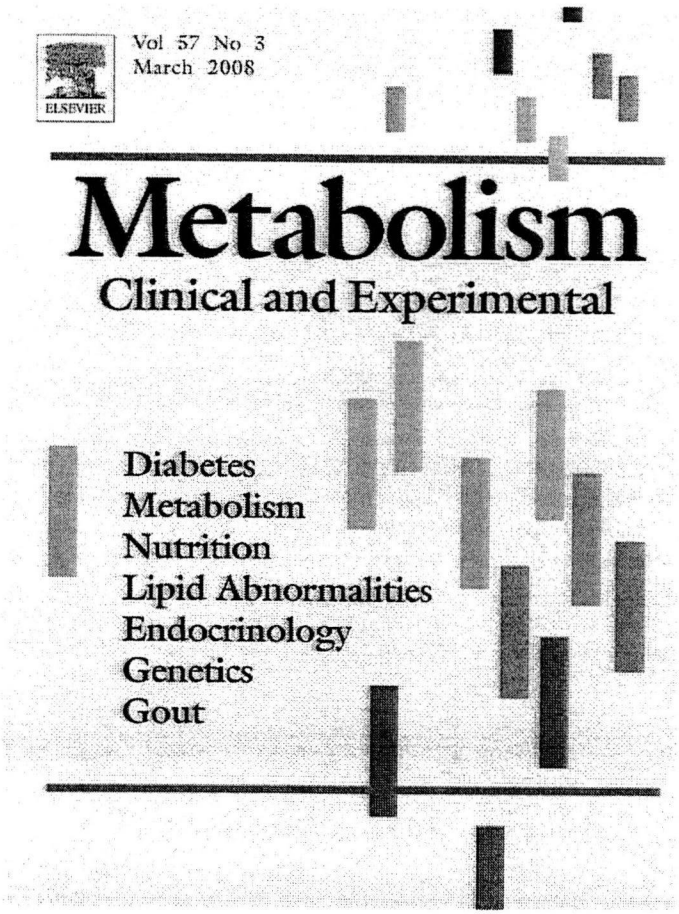

ORIGINAL ARTICLE

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Effects of metformin on peripheral insulin sensitivity and intracellular lipid contents in muscle and liver of overweight Japanese subjects

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Aim: Recent studies suggest that insulin resistance is associated with increased intrahepatic lipid (IHL) and intramyocellular lipid (IMCL) contents. While metformin improves insulin resistance mainly in liver, its effects on IHL and IMCL have not been clarified yet. The aim of this study was to investigate the effects of low-dose metformin (750 mg/day) on peripheral insulin sensitivity, IHL and IMCL.

Methods: Before and 3 months after low-dose metformin therapy, eight overweight/obese Japanese subjects [body mass index (BMI) >25 kg/m^2] were studied with blood sampling, measurement of IHL and IMCL by ^{1}H magnetic resonance spectroscopy and glucose infusion rate (GIR) during euglycaemic–hyperinsulinaemic clamp as an index of peripheral insulin sensitivity.

Results: A 3-month low-dose metformin therapy did not alter body weight, total body fat, fat distribution or physical activity level but increased GIR by 31% (from 6.24 ± 0.86 to 7.82 ± 0.82 mg/kg/min, $p < 0.01$). Although metformin treatment did not alter IMCL (from 4.1 ± 1.0 to 4.2 ± 0.9, not significant), it decreased IHL by 21% (from 15.9 ± 2.8 to 11.8 ± 2.2%, $p < 0.05$).

Conclusions: A 3-month low-dose metformin treatment improved peripheral insulin sensitivity and reduced IHL, without significantly changing BMI, adiposity or IMCL.

Keywords: biguanide, clinical study, insulin resistance, oral hypoglycaemic agent

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Introduction

Recent studies have focused on the relation between intracellular lipids and insulin resistance [1–3]. Cross-sectional studies using ^{1}H magnetic resonance spectroscopy (^{1}H-MRS) or biopsy specimens have demonstrated that intramyocellular lipid (IMCL) concentration correlates with peripheral insulin resistance [1–3]. On the other hand, hepatic insulin resistance correlated with intrahepatic lipid (IHL) content in both healthy subjects [2] and patients with type 2 diabetes [3].

Previous studies showed that moderate weight loss by calorie restriction reduces hepatic fat accumulation and improves glucose metabolism in the liver [4]. In addition, we showed that increased physical activity by exercise therapy reduces IMCL and improves insulin resistance in muscles [5]. Thus, accumulation of fat intracellularly in muscles and liver is associated with insulin resistance of the respective organ. Consequently, any intervention may affect the level of intracellular fat differently in different organs. Clinically, it is important to elucidate the precise mechanism of intervention on

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insulin resistance with relation to intracellular fat in liver and muscle.

Metformin is widely used in the treatment of type 2 diabetes; however, there is still a gap in our understanding of the exact mechanism of action of this agent. Most studies suggested that the primary target organ of hypoglycaemic effect of metformin is the liver [6]. On the other hand, it remains controversial whether metformin can improve peripheral insulin resistance [6]. It is probable that the different findings reported in the literature are mainly because of differences in patient selection and the dose and duration of metformin treatment.

At least in rats, metformin interferes with mitochondrial energy production, possibly through activation of AMP-activated protein kinase (AMPK) [7], which is accompanied by inhibition of lipogenesis and a decrease in hepatic steatosis. Consistent with this proposal, metformin decreased transaminase in patients with non-alcoholic fatty liver disease and non-alcoholic steatohepatitis [8–10]. On the other hand, Tiikkainen *et al.* [11] reported that metformin has no effect on hepatic fat content in type 2 diabetes. Thus, it is still not clear at this stage whether metformin can decrease IHL. Also, the discrepant findings on the effects of metformin on IHL are probably based on differences in patient selection and the dose and duration of metformin treatment.

While metformin treatment often results in moderate weight reduction mainly because of digestive disturbances [12,13], low-dose metformin induced less digestive disturbances [14,15]. Thus, the use of low-dose metformin could allow a better evaluation of the effect of metformin as it excludes the effect of body weight reduction. In this regard, the maximum dose of metformin that can be prescribed in Japan is 750 mg/day; this very low level was set in the 1950s and has not been revised since.

The present study was designed to investigate the effect of low-dose metformin (750 mg/day) in overweight/obese [body mass index (BMI) >25 kg/m^2] subjects, especially its effect on intracellular lipid. The results suggested that low-dose metformin therapy improves peripheral insulin resistance and fatty liver, independent of changes in body weight and fat distribution.

Materials and Methods

The Study Subjects

The study subjects were five men and three women. The entry criteria included age from 30 to 49 years, BMI > 25 kg/m^2 and stable body weight for at least 3 months before the study. All subjects were previously diagnosed as mild type 2 diabetes, with glycated haemoglobin (HbA_{1c}) <5.8% or impaired glucose tolerance based on 75 g oral glucose tolerance test. Patients with acute or chronic disease based on past history, physical examination and standard laboratory tests (blood cell counts, serum creatinine, electrolyte concentrations, liver function tests and ECG) were excluded. All subjects gave written informed consent to the study, which was approved by the Ethics Committee of Juntendo University. No subjects were taking any medications known to affect glucose metabolism.

Study Design

Subjects fasted overnight from 21:00 to 08:00 hours before baseline measurements. They underwent measurement of total body fat content by dual energy X-ray absorptiometry. IMCL in the right tibialis anterior (TA) muscle and IHL of segment 6 in the liver were measured by ^{1}H-MRS. In addition, intra-abdominal and subcutaneous fat were measured by MRI (VISART EX V4.40; Toshiba, Tokyo, Japan). Peripheral insulin sensitivity was evaluated by the euglycaemic–hyperinsulinaemic clamp method [16]. The mean physical activity level was estimated with an ambulatory accelerometer before and during intervention (Lifecorder; Suzuken, Nagoya, Japan) [5]. After baseline evaluation, all subjects received 500 mg/day metformin therapy for 4 weeks, which was followed by 750 mg/day metformin treatment for 8 weeks. All subjects were instructed to continue their dietary intake and physical activity during the intervention.

Biochemical Tests

Serum lipids (total cholesterol, HDL cholesterol, LDL cholesterol, free fatty acid and triglyceride) and liver function tests [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase (γ-GTP)] were measured by enzymatic methods and ultraviolet methods respectively (SRL, Tokyo, Japan). Plasma insulin and leptin concentrations were determined by radioimmunoassay (LINCO Research, MO, USA). Measurements of serum concentrations of adiponectin, intercellular adhesion molecule (ICAM)-1 and plasminogen activator inhibitor-1 (PAI-1) by enzyme-linked immunosorbent assays were outsourced to a private laboratory (SRL and Otsuka Pharmaceuticals, Tokyo, Japan). The level of high-sensitivity C-reactive protein (hs-CRP) was measured by latex nephelometry at SRL Laboratory. Homoeostasis model assessment of insulin resistance (HOMA-IR) was calculated as the product of fasting plasma insulin (μU/ml) and fasting plasma glucose level (mg/dl) divided by 405.

Proton Magnetic Resonance Spectroscopy

IMCL and IHL were measured at 08:00 hours as described previously [3,5,17–19]. Briefly, IMCL of the right TA muscle and IHL of segment 6 in the liver were measured by ^{1}H-MRS using a knee coil and a whole body coil respectively (VISART EX V4.40; Toshiba). Voxels ($1.2 \times 1.2 \times 1.2$ cm^3 for TA and $2 \times 2 \times 2$ cm^3 for the liver) were positioned in the TA muscle or liver, avoiding visible interfascial fat and blood vessels, and the voxel sites were carefully matched at each examination. Imaging parameters were set as follows: repetition time, 1500 ms; echo time, 136 ms (TA) or 10 ms (liver); acquisition numbers, 192 for TA and 8 for liver and 1024 data points over a 1000-kHz spectral width. After examination, resonances were quantified by reference to the methylene signal intensity (S-fat), with peaks being observed at ~1.25 p.p.m. in TA and at ~1.3 p.p.m. in the liver. IMCL was quantified by S-fat and the creatine (Cre) signal at 3.0 p.p.m. as the reference, and was calculated as a ratio relative to Cre (S-fat/Cre). IHL was quantified by S-fat and H_2O at ~4.7 p.p.m. as the internal reference and calculated as a percentage of H_2O + S-fat [S-fat $\times$ 100/(H_2O + S-fat)] as described previously [3]. In lean Japanese healthy subjects, mean IMCL and IHL levels were 2.2 ± 0.3 (n = 16) and 2.2 ± 0.5 (n = 9) respectively. The mean coefficient of variation for the calculated IMCL and that for IHL are 8.16% (n = 4) and 4.50% (n = 4) respectively.

Intra-abdominal and Subcutaneous Fat

Intra-abdominal and subcutaneous fat were measured as described previously using MRI [20]. Briefly, T1-weighted transaxial scans were obtained to determine intra-abdominal and subcutaneous fat in a region extending from 8 cm above to 8 cm below the fourth and fifth lumbar interspaces (16 slices; field of view, 370×400 mm^2; slice thickness, 10 mm; breath-hold repetition time, 6000 ms; echo time 78 ms). Intra-abdominal and subcutaneous fat areas were measured using an image analysis program (MEDx; L.A. Systems, Tochigi, Japan). The intensity of the nadir between the lean mass and fat peaks was determined by histogram of pixel intensity and used as a cut point. Intra-abdominal and subcutaneous adipose tissue areas were calculated as described previously [20].

Euglycaemic–Hyperinsulinaemic Clamp

Patients were fasted overnight from 21:00 to 08:00 hours before each test. On the study day, with the use of artificial endocrine pancreas (STG 22; Nikkiso, Shizuoka, Japan), euglycaemic–hyperinsulinaemic clamp (target plasma glucose of 95 mg/dl and insulin infusion rate of 100 mU/m^2/min) was applied to determine insulin sensitivity in peripheral tissue, as described previously [5]. The steady-state glucose infusion rate (GIR) was monitored from 105 to 120 min, and the mean GIR during that period was used as a marker of peripheral insulin sensitivity.

Statistical Analysis

All data are expressed as mean ± s.e.m. Baseline data were compared with those obtained after treatment by the paired *t*-test. Statistical significance was set at $p < 0.05$.

Results

Table 1 shows the clinical characteristics of eight study subjects before and 3 months after the treatment. The mean age of the subjects was 34.8 ± 2.7 years. All subjects completed this study without evident untoward effects. As we anticipated, low-dose metformin treatment did not alter body weight. Furthermore, no changes in total body fat, abdominal visceral and subcutaneous fat and daily physical activity were observed throughout the study. In addition, metformin treatment did not result in significant changes in fasting plasma glucose, insulin, HOMA-IR, HbA_{1c}, total cholesterol, HDL cholesterol, triglyceride, AST, ALT and γ-GTP. No changes in adipocytokines (leptin and adiponectin), total PAI-1, hs-CRP and ICAM-1 were observed throughout the treatment (Table 1).

On the other hand, the steady-state GIR, which mainly reflects glucose uptake in skeletal muscles, significantly increased by 31% after metformin treatment (from 6.24 ± 0.86 to 7.82 ± 0.82 mg/kg/min, $p < 0.01$). With regard to intracellular fat, metformin treatment significantly decreased IHL by 21% (from 15.9 ± 2.8 to 11.8 ± 2.2%, $p < 0.05$) but not IMCL (from 4.1 ± 1.0 to 4.2 ± 0.9, not significant) (figure 1). The percent change of IHL was not correlated to the percent change of GIR ($r = -0.614$, not significant).

Discussion

The present study evaluated the effects of a 3-month low-dose metformin therapeutic regimen in obese subjects. The regimen improved peripheral insulin sensitivity and decreased IHL without affecting IMCL. These changes were not accompanied by reduction of body weight.

Table 1 Clinical parameters at baseline and after 3-month low-dose metformin treatment

	Baseline	End of study
Body weight (kg)	84.0 ± 5.3	83.9 ± 5.3
Body mass index (kg/m^2)	30.5 ± 1.9	30.5 ± 2.0
Body fat (%)	33.4 ± 2.7	33.9 ± 2.9
Physical activity (kcal/day)	324 ± 36	304 ± 23
Fasting plasma glucose (mg/dl)	103 ± 4	102 ± 5
Fasting plasma insulin (μU/ml)	19.6 ± 5.4	21.2 ± 5.1
Homoeostasis model assessment of insulin resistance [(mg/dl)(μU/ml)/405]	4.3 ± 1.0	5.7 ± 1.8
Glycated haemoglobin (%)	5.1 ± 0.1	5.1 ± 0.1
Total cholesterol (mg/dl)	200 ± 15	199 ± 12
HDL cholesterol (mg/dl)	49 ± 3	48 ± 3
Triglyceride (mg/dl)	200 ± 30	198 ± 39
Free fatty acid (mmol/l)	0.68 ± 0.13	0.69 ± 0.17
Aspartate aminotransferase (IU/l)	34 ± 7	29 ± 6
Alanine aminotransferase (IU/l)	59 ± 22	51 ± 19
γ-Glutamyltransferase (IU/l)	91 ± 36	87 ± 35
Leptin (ng/ml)	12.4 ± 3.8	14.1 ± 5.9
Adiponectin (μg/ml)	4.7 ± 0.3	5.0 ± 0.4
High-sensitivity C-reactive protein (mg/l)	2.06 ± 0.84	2.29 ± 0.91
Total plasminogen activator inhibitor-1 (ng/ml)	57 ± 14	40 ± 9
Intercellular adhesion molecule-1 (ng/ml)	245 ± 25	270 ± 30
Abdominal visceral fat (cm^3)	1634 ± 268	1567 ± 333
Abdominal subcutaneous fat (cm^3)	2890 ± 412	2921 ± 501

Data are mean ± s.e.m. of eight subjects.

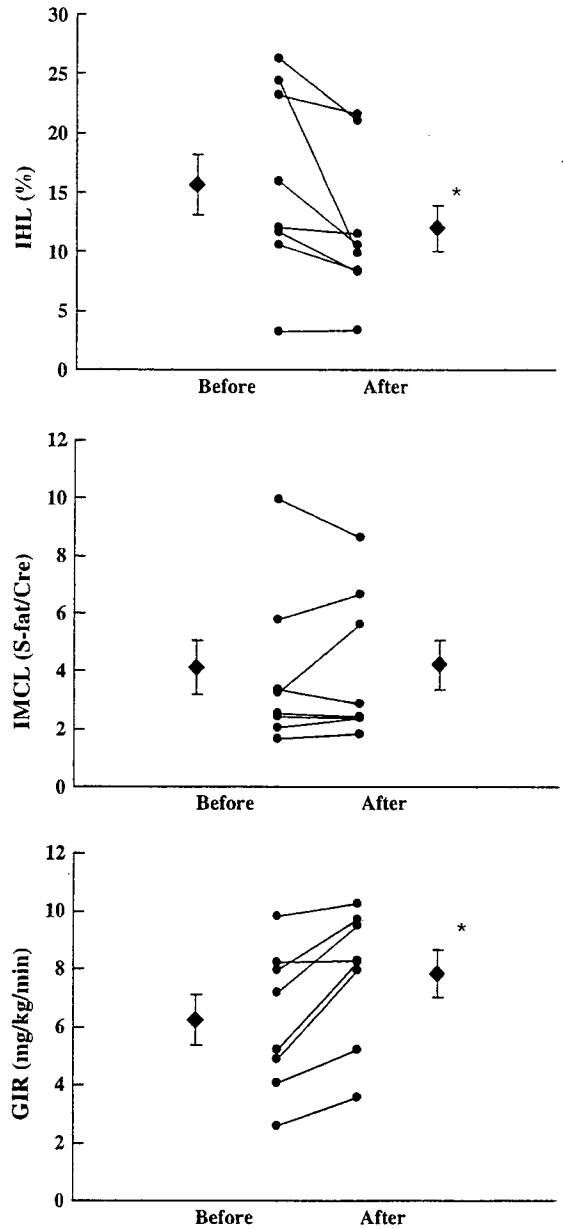

Fig. 1 Individual and mean changes in glucose infusion rate (GIR), intramyocellular lipid (IMCL) and intrahepatic lipid (IHL). *$p < 0.05$ compared with baseline (before treatment).

Previous studies demonstrated that metformin treatment with lifestyle modification significantly improved ALT and reduced body weight [9,10]. However, ALT levels do not necessarily reflect those of IHL [21]. In addition, previous studies demonstrated that subtle body weight reduction by calorie restriction could efficiently decrease IHL [4,5]. Thus, the effect of metformin on IHL beyond body weight–lowering effect has not been fully elucidated yet. In the present study, we investigated the effect of metformin on fat in liver. We evaluated IHL level by ^{1}H-MRS, which provides precise measurement of triglyceride levels in the liver [17]. In addition, we asked patients not to change their physical activity during the study, which was checked by accelerometer because high habitual physical activity likely decreases IHL [22]. Furthermore, we used low-dose metformin, which has less gastrointestinal discomfort [14], to exclude the effect of body-weight reduction on fatty liver. As we anticipated, low-dose metformin did not change BMI and total fat amount. However, metformin decreased IHL by 22%. Although it has been reported that metformin has no effect on hepatic fat content in type 2 diabetes [11], our results clearly showed that low-dose metformin therapy reduces IHL level independent of body weight reduction in Japanese overweight subjects. Our finding is more convincing, considering the fact that metformin increases AMP kinase activity in the liver, which enhances fat oxidation and inhibits *de novo* lipogenesis [7]. Probably, the different findings were based on differences in patient selection. In the

previous study [11], the study subjects were patients with type 2 diabetes whose HbA_{1c} was 6.9%, while those of this study were patients with mild type 2 diabetes or impaired glucose tolerance whose mean HbA_{1c} was 5.1%. It is possible that baseline glycaemic level might influence the effect of metformin on IHL, although the mechanism is unknown. In fact, several previous studies showed that metformin successfully improved fatty liver and/or liver function test in the subjects without diabetes.

The major hypoglycaemic effect of metformin is achieved through improvement of hepatic insulin resistance [23,24]. Because hepatic insulin resistance correlates well with IHL both in healthy subjects [2] and in patients with type 2 diabetes [3], the reduction of IHL probably results in the reduction of hepatic glucose production, although we did not assess it. On the other hand, in isolated human muscle cells, metformin increased glucose uptake [25,26]. In addition, metformin treatment increased AMPK activity in skeletal muscles of patients with type 2 diabetes, which was associated with higher rates of glucose disposal and muscle glycogen concentration [23]. Thus, it is reasonable that metformin treatment results in increase in GIR. On the other hand, in the present study, the increase in GIR was not accompanied by reduction of IMCL, although activation of AMPK might result in reduction of IMCL. We do not know the exact reason of this finding. Probably, the dose used in this study results in improvement of glucose uptake without reduction of IMCL.

Metformin therapy improved peripheral insulin sensitivity but did not alter HOMA-IR. Although HOMA-IR is significantly correlated to peripheral insulin sensitivity measured by euglycaemic–hyperinsulinaemic clamp, the correlation coefficient was reported from 0.58 to 0.88 [27]. Because euglycaemic–hyperinsulinaemic clamp method is regarded as a more sensitive method to detect insulin sensitivity than HOMA-IR, the small improvement of peripheral insulin sensitivity observed in this study might not be detected by HOMA-IR. Similar to this study, a previous report showed that metformin improved liver function and glucose disposal rate during euglycaemic–hyperinsulinaemic clamp in non-alcoholic steatohepatitis, while fasting glucose and insulin levels were not significantly affected [8].

In conclusion, a 3-month low-dose metformin regimen significantly improved peripheral insulin sensitivity and reduced IHL in obese Japanese subjects. In diabetes prevention programme, metformin prevents the onset of diabetes mainly in subjects with high BMI [28], which is a condition associated with fatty liver. Considering the good relationship between hepatic insulin resistance and IHL, this effect seems to be involved in the prevention of the onset of diabetes.

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ORIGINAL ARTICLE

Comparative study of the cardio-ankle vascular index and ankle–brachial index between young Japanese and Mongolian subjects

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Mongolian people have higher mortality and morbidity rates due to cardiovascular disease (CVD) than Japanese people. The cardio-ankle vascular index (CAVI) and ankle–brachial index (ABI) are both atherosclerosis-related indexes. Presently, there is no comparative information on CAVI and ABI among young subjects between Mongolian and Japanese people. A total of one hundred Mongolian (men: 39%, mean age: 20.9 ± 2.2 years) and 115 Japanese volunteers (men: 39%, mean age: 22.0 ± 1.8 years) were recruited from among university students. The body mass index (BMI), heart rate (HR), blood pressure (BP), CAVI, ABI, carotid intima–media thickness, blood total cholesterol (TC), glucose and C reactive protein levels were measured. The levels of BMI, HR and diastolic BP were significantly higher, and TC and glucose were significantly lower in the Mongolian subjects than in the Japanese subjects. The CAVI values (median (interquartile range): 6.5 (5.8–7.0) *vs.* 5.6 (5.2–6.0)) and ABI (1.11 (1.05–1.17) *vs.* 1.09 (1.05–1.15)) were significantly higher in the Mongolian subjects than in the Japanese subjects. The patterns of correlation between CAVI, ABI and other atherosclerotic parameters were different: in age-, gender- and BMI-adjustment correlation tests for CAVI and ABI, HR ($r=-0.25$ for CAVI and ABI) showed a correlation in the Mongolian subjects, and for ABI systolic BP ($r=-0.28$) showed a correlation in the Japanese subjects. These results suggest that Mongolian subjects may be at higher risk of CVD, even among younger individuals, than Japanese subjects.
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Keywords: CAVI; ABI; young people; lifestyle; altitude

INTRODUCTION

Cardiovascular disease (CVD) is a major world-wide cause of premature death.[1] Since 1995, CVD has been increasing and has become a more significant health issue than cancer in Mongolia.[2] The WHO statistical data in 2005 suggested that the Mongolian people had a shorter life expectancy by 17 years in comparison with the Japanese people.[3] In the country health information profiles of the WHO report in 2006, the mortality rates per 100 000 of the population were 4.5 and 17.3 due to hypertension, 21.3 and 43.7 due to ischemic heart disease, and 246.3 and 230.6 due to cerebrovascular diseases in Japan and Mongolia, respectively.[3] Therefore, comparative studies are necessary to determine the atherosclerotic factor traits between Mongolian subjects and those from countries such as Japan, which has a very high average lifespan.

One of the crucial issues to prevent CVD is the detection of atherosclerotic alterations among asymptomatic subjects. The cardio-ankle vascular index (CAVI) and ankle–brachial index (ABI) are both atherosclerosis-related indexes that are particularly reflective of arterial stiffness and stenosis, respectively.[4,5] In fact, both indexes are associated with atherosclerotic risk factors and predict the future of CVD.[4,5] CAVI is a new parameter, independent of blood pressure (BP), and the clinical significance of CAVI for CVD has been established recently.[6] ABI is also useful for the diagnosis of low extremity peripheral arterial stenotic disease.[7]

Age, male gender, smoking, lack of exercise, obesity, dyslipidemia, diabetes and hypertension are routinely considered to be well-known atherosclerotic risk factors.[8,9] Although there have been a few comparative studies reporting higher levels of body mass index (BMI) and BP in Mongolian subjects in comparison with Japanese subjects,[10,11] the earlier studies have not examined young subjects. To prevent atherosclerosis in the long term, it is necessary to start the prevention in the early stages of the condition in young people. However, no information on atherosclerosis-related parameters such as CAVI and ABI is available for young persons. Although the difference in lifestyle factors between Mongolian and Japanese people can also affect the status of atherosclerotic risk factors, there have been few comparative

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studies on regulation of lifestyle factors. Therefore, the objective of this study was to examine the atherosclerotic parameters, including CAVI and ABI, in consideration of some lifestyle factors in young Mongolian and Japanese subjects.

MATERIALS AND METHODS

A total of 100 Mongolian volunteers (men: 39, women: 61), aged 18–25 years, were recruited from the Health Sciences University of Mongolia (Ulaanbaatar city, Mongolia). Similarly, 115 volunteers (men: 45, women: 70) were recruited from the Jichi Medical University (JMU) in Tochigi Prefecture, Japan. The participants' data were collected under the same conditions (that is, during a regular class period without any examinations, or at room temperature) between June 2006 and January 2008. All eligible subjects were asymptomatic without any known history of cardiovascular, cerebrovascular, kidney or liver disease. None of the subjects were currently consuming alcohol. This study was approved by the Ethical Committee of JMU and the Mongolian Ministry of Health, and each volunteer gave informed consent.

With respect to lifestyle factors, questionnaires on meat intake (categorized by $\geqslant 4$ times in a week or <4 times in a week), vegetable intake ($\geqslant 4$ times in a week or <4 times in a week), salt intake (high or low), smoking (current or former/never) and physical activity ($\geqslant 1$ time in a week or <1 time a week) were self-administered. The BMI was calculated as weight divided by the square of body height while wearing light clothes. Serum samples were collected from the antecubital vein after a 12-h overnight fast and the samples were stored at −80 °C. Blood measurements were performed at a laboratory facility in Japan. The serum total cholesterol and plasma glucose were measured enzymatically, and C reactive protein was measured using a latex agglutination immune assay (EIKEN Chemical Co. Ltd, Tokyo, Japan).

Oscillometric technology was applied to evaluate the heart rate (HR), CAVI and ABI using a VaSera VS-1000 vascular screening system (Fukuda Denshi Co. Ltd, Tokyo, Japan). The volunteers were made to lie on a bed in supine position for at least 10 min, as described earlier.[12] The CAVI values were obtained from the brachial and ankle pulse wave forms, systolic and diastolic BP (SBP and DBP),[13] which were measured by using a cuff suitable for each subject's arm according to the proposed technical manual.[14] The stiffness parameter was calculated using the formula $CAVI=\ln(Ps/Pd)\times 2\rho/\Delta P\times PWV^2$ (where Ps is the SBP, Pd the DBP, ρ the blood density, ΔP the pulse pressure and PWV the pulse wave velocity between the aortic value and the ankle). ABI was determined based on the SBP in both the upper (brachial arterial) and lower (tibial arterial) BP.[5] ABI was calculated by dividing the ankle SBP by the brachial SBP. The SBP and DBP were measured for each subject's brachial artery: although this was a single measurement, the subjects showing hypertensive levels were not detected.

Moreover, after 5 min of relaxing in the supine position, the intima–media thickness (IMT) of the bilateral common carotid arteries of the volunteers was measured by ultrasonography (EH54-9DR system, DIASUS, Scotland, UK). The mean IMT levels were determined by the average of the values at points 1, 2 and 3 cm below the carotid bifurcation on each side of artery.

Statistical analysis

The data are presented as the mean ± s.d.; in cases of parameters with non-normal distributions (BMI, HR, SBP, DBP, CAVI, ABI, IMT, total cholesterol, glucose and C reactive protein), the data are shown as the median (interquartile range). In all analyses, parameters with non-normal distributions were used after log-transformations. All statistical analyses used the Statistical Package for Social Science (SPSS) version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). A P-value <0.05 was considered to be statistically significant. Comparisons between groups were examined using Student's t-test (for continuous variables) and the χ^2-test/Fisher's exact probability test (for categorical variables). As the age and BMI were significantly different between the Japanese and Mongolian subjects, age and gender were treated as co-variables in the comparison with BMI, and subsequently, in the comparison of other parameters, age, gender and BMI were treated as co-variables. To analyze the within-lifestyle-categorized group differences and between-race-based group differences in the relationship of lifestyle factors with atherosclerotic parameters, age, gender, BMI and other lifestyle factors were treated as co-variables. To investigate the correlations of CAVI and ABI with other atherosclerotic parameters, Pearson's correlation and partial correlation tests were used with adjustments for age, gender and BMI.

RESULTS

The mean age was slightly but significantly older in the Japanese subjects than in the Mongolian subjects. In the comparative data for the atherosclerotic parameters between the young Japanese and Mongolian subjects (Table 1), the BMI in the Mongolian subjects was significantly higher than that in the Japanese subjects. The HR and DBP levels were significantly higher and SBP levels tended to be high in the Mongolian subjects. CAVI and ABI were significantly higher in the Mongolian subjects than in the Japanese subjects. The total cholesterol and glucose levels were significantly lower in the Mongolian subjects than in the Japanese subjects, but the IMT and C reactive protein levels were similar.

Regarding the correlations of CAVI and ABI to other atherosclerotic parameters in the respective race-based groups (Table 2), a simple correlation test between bivariables (model 1) showed that CAVI was positively correlated with age in the Mongolian subjects. However, a correlation test with adjustments for age, gender and BMI (model 2) clearly showed a negative correlation between CAVI and HR in the Mongolian subjects. In contrast, in both correlation models of ABI, HR was correlated negatively with ABI in the Mongolian subjects and SBP was negatively correlated with ABI in the Japanese subjects.

Regarding lifestyle factors (Table 3), the Mongolian subjects had a higher percentage of meat intake and current smoking, in comparison with that in the Japanese subjects. Other lifestyle factors did not show significant differences. As for the relationship between the lifestyle factors and atherosclerotic parameters (Table 4), both differences between the race-based groups and differences in lifestyle categories within the respective race-based groups were observed. Only vegetable and salt intake showed significant between-group differences in some atherosclerotic parameters: Mongolian subjects with lower vegetable intake levels had significantly higher levels of HR than those with higher vegetable intake levels. Japanese subjects with high salt intake had significantly higher levels of HR than those with low salt intake. In contrast, the SBP was significantly higher in subjects with low salt

Table 1 Comparison of atherosclerotic parameters between young Japanese and Mongolian subjects

Parameters	*Japanese (n=115)*	*Mongolian (n=100)*
Age (years)	22.0 ± 1.8	20.9 ± 2.2**
Men (%)	39	39
BMI (cm/kg^2)	20.5 (19.3–22.3)	21.4 (19.8–23.3)*
HR (b.p.m.)	57.0 (50.3–65.0)	65.0 (61.0–70.0)**
SBP (mm Hg)	111.0 (105.0–120.8)	114.0 (108.0–121.8)
DBP (mm Hg)	65.0 (62.0–68.5)	70.3 (65.1–76.4)*
CAVI	5.6 (5.2–6.0)	6.5 (5.8–7.0)**
ABI	1.09 (1.05–1.15)	1.11 (1.05–1.17)*
IMT (mm)	0.42 (0.37–0.47)	0.44 (0.38–0.50)
TC (mmol l^{-1})	4.40 (4.04–5.06)	3.99 (3.55–4.38)**
Glucose (mmol l^{-1})	4.88 (4.61–5.16)	4.22 (3.90–4.50)**
CRP (mg per 100 ml)	0.03 (0.01–0.05)	0.04 (0.02–0.08)

Abbreviations: BMI, body mass index; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; CAVI, cardio-ankle vascular index; ABI, ankle–brachial index; IMT, intima–media thickness; TC, total cholesterol; CRP, C reactive protein.
Only age is presented as mean ± s.d. Other parameters are presented as the median (interquartile range).
Significance level: *$P<0.05$, **$P<0.001$ (age: Student's t-test, gender: χ^2-test, other parameters: Student's t-test after log-transformation; BMI: age and gender adjustments are performed, other parameters: age, gender and BMI adjustments are performed).

Table 2 Correlation of the CAVI and ABI with other atherosclerotic parameters in young Japanese and Mongolian subjects

	CAVI		*ABI*	
	Japanese	*Mongolian*	*Japanese*	*Mongolian*
Model 1				
Age (years)	0.14 (0.1)	0.35 (<0.001)**	0.15 (0.1)	0.15 (0.1)
Gender (men)	0.08 (0.4)	−0.12 (0.2)	−0.21 (0.03)*	0.14 (0.2)
HR (b.p.m.)	0.002 (1.0)	−0.16 (0.1)	−0.03 (0.8)	−0.23 (0.02)*
SBP (mm Hg)	0.05 (0.6)	−0.16 (0.1)	−0.32 (<0.001)**	−0.05 (0.6)
DBP (mm Hg)	0.08 (0.4)	0.06 (0.9)	−0.02 (0.8)	−0.04 (0.7)
IMT (mm)	0.04 (0.7)	−0.12 (0.2)	−0.01 (0.9)	0.12 (0.2)
TC (mmol l^{-1})	0.07 (0.5)	0.06 (0.6)	0.10 (0.3)	0.02 (0.8)
Glucose (mmol l^{-1})	0.02 (0.8)	−0.08 (0.4)	−0.01 (1.0)	0.08 (0.4)
CRP (mg per 100 ml)	0.06 (0.6)	−0.16 (0.1)	0.11 (0.3)	0.16 (0.1)
Model 2				
HR (b.p.m.)	−0.04 (0.7)	−0.25 (0.01)*	−0.13 (0.2)	−0.25 (0.02)*
SBP (mm Hg)	0.09 (0.4)	−0.15 (0.2)	−0.28 (0.006)*	−0.13 (0.2)
DBP (mm Hg)	0.12 (0.2)	0.001 (1.0)	0.01 (0.9)	−0.02 (0.8)
IMT (mm)	0.04 (0.7)	0.04 (0.7)	0.06 (0.6)	0.17 (0.1)
TC (mmol l^{-1})	0.12 (0.2)	0.001(1.0)	0.09 (0.3)	−0.00 (1.0)
Glucose (mmol l^{-1})	−0.001(0.9)	−0.07 (0.5)	−0.002 (1.0)	0.06 (0.6)
CRP (mg per 100 ml)	0.09 (0.4)	0.10 (0.4)	−0.08 (0.4)	0.10 (0.4)

Abbreviations: HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; ABI, ankle–brachial index; CAVI, cardio–ankle vascular index; IMT, intima-media thickness; TC, total cholesterol; CRP, C reactive protein.
Data are presented as *r* (*P*-value).
Model 1: simple correlation between the parameters. Model 2: partial correlation between the parameters with the adjustments for age, gender and BMI.
Significance level: **P*<0.05, ***P*<0.001.

intake than in Mongolian subjects with high salt intake. No significant relationships between CAVI, ABI and lifestyle factors were observed in either the Mongolian or the Japanese subjects.

DISCUSSION

This study is the first to compare CAVI and ABI between young Japanese and Mongolian subjects considering lifestyle factors. The overall levels of atherosclerotic parameters were shown to be higher, despite the lower levels of total cholesterol and glucose in Mongolian subjects than those in Japanese subjects. In particular, BMI, HR, DBP, CAVI and ABI levels were significantly higher in the Mongolian subjects. Although higher levels of BMI and BP in Mongolian subjects have been noted in earlier studies,[10,11] those studies did not examine young people. The finding of higher levels of atherosclerotic parameters in Mongolian subjects in comparison with Japanese subjects cannot be ignored when these are present even in young subjects, if these findings are relevant to the higher rate of CVD in the Mongolian people.

In addition, the non-remarkable influences of lifestyle factors and similar levels (within reference ranges) on IMT and C reactive protein between Mongolian and Japanese subjects suggest that the present study population showed only a weak effect of their accumulated daily lifestyles, and that the subjects were free from atherosclerotic changes, probably because the population comprised young and undiseased subjects. Accordingly, the present data might be a valuable reference to observe the prospective incidence of CVD.

CAVI is a new marker for the stiffness of the aorta, femoral and tibial arteries, independently of BP.[15] CAVI was higher in Mongolian subjects. Aorta stiffness was not expected in this young population, but these results imply the presence of promoting factors for arterial stiffness in Mongolian subjects. Interestingly, in the confounder-adjustment tests CAVI was significantly correlated with HR in Mongolian subjects, whereas a negative relationship between HR and CAVI has not been documented earlier. In contrast, ABI is shown to be a highly sensitive marker to diagnose peripheral arterial

Table 3 Comparison in lifestyle factors between young Japanese and Mongolian subjects

Lifestyle category	*Japanese*	*Mongolian*
Meat intake		
⩾4 times in a week	15 (13.0)	47 (47.0)**
<4 times in a week	100 (11.5)	53 (53.0)
Vegetable intake		
⩾4 times in a week	82 (71.2)	80 (80.0)
<4 times in a week	33 (28.7)	20(20.0)
Salt intake		
High	14 (12.2)	20 (20.0)
Low	101 (87.8)	80 (80.0)
Smoking		
Current	2 (2.6)	19 (19.0)**
Former/never	113 (97.4)	81 (81.0)
Physical activity		
⩾1 time in a week	74 (64.3)	62 (62.0)
<1 time in a week	41 (35.7)	38 (38.0)

Data are represented as number (%).
Significance level: **P*<0.05, ***P*<0.001 (χ^2-test or regarding smoking only Fisher's exact probably test).

Table 4 Relationship between lifestyles and atherosclerotic parameters

	Japanese			*Mongolian*			
	Q_0	Q_1	P_1	Q_0	Q_1	P_2	P_3
Vegetable intake	*n*=82	*n*=33		*n*=80	*n*=20		
HR	57.0 (50.8–65.3)	57.0 (49.5–63.5)	NS	65.0 (61.0–69.8)	65.0 (62.0–70.0)	0.01	<0.0001
Salt intake	*n*=101	*n*=14		*n*=80	*n*=20		
HR (b.p.m.)	56.0 (49.8–64.0)	65.5 (58.5–69.0)	0.02	64.5 (61.3–69.8)	67.0 (59.5–76.0)	NS	<0.001
SBP (mm Hg)	112.3 ± 11.0	108.9 ± 7.3	NS	114.0 (108.0–123.0)	115.0 (106.8–119.0)	0.02	0.02

Abbreviations: HR, heart rate; NS, no significance; SBP, systolic blood pressure. Data are presented the mean ± s.d. or median (interquartile range). Vegetable intake: Q_0= ≥ 4 times in a week, Q_1= < 4 times in a week. Salt intake: Q_0=less salty, Q_1=salty.
P_1: within-group difference in Japanese subjects. P_2: within-group difference in Mongolian subjects. P_3: group difference between Japanese and Mongolian subjects.
Significance level: $P<0.05$ (all analyses were performed with the adjustments for age and gender, BMI and other lifestyle factors.

stenotic disease, but it was not expected in this study population, because all values of ABI did not correspond to the disease criteria level (of <0.9).[16] In this situation, higher ABI levels were found in the Mongolian subjects in comparison with the Japanese subjects. The absence of a significant difference in SBP (brachial BP) between Mongolian and Japanese subjects suggests that the ankle BP might affect ABI levels to greater extent. Interestingly, HR was correlated negatively with ABI in the Mongolian subjects, which is consistent with earlier findings.[17,18] In the Japanese subjects, the SBP was negatively correlated with ABI, which is also consistent with the earlier results.[19]

Thus, although differences in the correlation pattern of CAVI and ABI with other atherosclerotic parameters between young Mongolian and Japanese subjects were identified, the reasons are unclear. As a possible mechanism, the residential altitude is very different between Japanese and Mongolian people and it has been documented that the altitude can contribute to circulatory characteristics such as HR and BP.[20,21] The mean altitude of JMU (Tochigi prefecture) is approximately 50 m (165 feet) and that of Ulaanbaatar is 1350 m (4429 feet) above sea level. In our study, the higher levels in HR and BP in the Mongolian subjects may be explained by the earlier study results that HR and BP (DBP in particular) increased with rising altitude levels.[20,21] However, basically CAVI is independent of BP (at least, the direct causes of increases in BP cannot necessarily be similar to those in CAVI). Importantly, one study has suggested that CAVI levels are higher in residents living at high altitude than those at sea level, and increases in hematocrit and oxidative stress by hypoxia are speculated as the causes.[21] In fact, the increased hematocrit levels in Mongolian subjects have already been reported.[20] Therefore, the results of higher CAVI levels in the Mongolian subjects may be partly due to such hypoxia-related factors. In addition, the higher ABI levels obtained in the Mongolian subjects may be partly explained by the fact that sympathetic nerve activity (in daily life) at high altitude also increases peripheral BP,[22] leading to a relative increase in the ankle BP to brachial BP. Therefore, the characteristics of atherosclerotic parameters in Mongolian subjects could be associated with the altitude level at which they live.

Moreover, although the genetic backgrounds are generally thought to be similar between Mongolian and Japanese people,[23] the frequencies of gene polymorphisms might be partly different.[24,25] For instance, a similar frequency of Japanese (8.8%) and Mongolian (9.9%) people having the Di (a) antigens is called the Mongoloid factor,[23] but some of the gene polymorphisms are different (for example, apolipoprotein ε4 allele and interleukin 8-251 A/T polymorphism).[24,25] Therefore, undetermined genetic factors contributing to the atherosclerotic parameters, especially CAVI and ABI, might explain some of the results.

This study had an advantage in that all measurements were performed using the same methodology and at the same facility, which seems to be important in comparative studies between countries. There are also a few limitations. Although the questionnaire generally covered the representative lifestyles relating to atherosclerosis, the categorization was simple and the detailed content of lifestyles was not confirmed. This could lead to difficulty in detecting the influences of lifestyle factors on atherosclerotic parameters. Although the sample size was small, the fact that the study population consisted of persons who got into the universities at a nationwide level in the respective countries strengthened the generalization of the results. The measures of hematocrit level, oxidative stress, sympathetic nerve activity and genetic polymorphisms were not examined, although these measures may provide further information on the altitude–atherosclerotic connection. Therefore, a study with a larger sample size and more detailed lifestyle-related questionnaires and the measurements of various biomarkers is a future challenge. Furthermore, other undetermined factors, for example, environmental factors other than altitude, should also be considered.

In conclusion, this study found that CAVI and ABI, in addition to BMI, HR and DBP, were significantly higher in the Mongolian subjects than in the Japanese subjects. These findings suggest that Mongolian people may be at higher risk of CVD, even among the younger generation. Further studies are needed to clarify the bio-physiological mechanism of higher levels of atherosclerotic parameters.

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