Comparison of plasma levels of obesity-related biomarkers among Japanese populations in Tokyo, Japan, São Paulo, Brazil, and Hawaii, USA

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Although Japanese in Japan and the USA are high-risk populations for colorectal cancer, the prevalence of obesity, one of the established risk factors for this disease, is low in these populations compared with other high-risk populations. To understand this inconsistency, we compared plasma obesity-related biomarkers in cross-sectional studies carried out in Tokyo, São Paulo, and Hawaii. We measured plasma levels of insulin-like growth factor-I (IGF-I), insulin-like growth factor-binding protein (IGFBP)-1, IGFBP-3, C-peptide, adiponectin, leptin, tumor necrosis factor-α, and interleukin-6 by immunoassay and total C-reactive protein, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides using a clinical chemistry autoanalyzer. A total of 299 participants were included in the present analysis, comprising 142 Japanese in Tokyo, 79 Japanese Brazilians in São Paulo, and 78 Japanese Americans in Hawaii. We found significantly lower plasma levels of C-peptide and IGF-I in Japanese in Tokyo than in Japanese Americans, and lower levels of leptin and triglycerides and higher levels of adiponectin, IGFBP-3, and high-density lipoprotein cholesterol in Japanese in Tokyo than in the other two populations. We also observed a significantly higher plasma IGFBP-1 level in Japanese Brazilians, and lower plasma levels of total cholesterol and low-density lipoprotein in Japanese Americans than in the other two populations. We observed significant differences in obesity-related biomarkers between the three Japanese populations. If our results are confirmed, the risk of colorectal cancer predicted on the basis of these biomarkers would be lowest for Japanese in Tokyo, followed by Japanese Brazilians and Japanese Americans. *European Journal of Cancer Prevention* 00:000–000 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

Introduction

Overweight and obesity are major public health concerns. Globally, the mean BMI has been increasing since 1980 (Finucane *et al.*, 2011). The worldwide age-standardized prevalence of obesity was 9.8% in men and 13.8% in women in 2008, which were nearly twice the rates in 1980 (4.8% for men and 7.9% for women) (Finucane *et al.*, 2011). In Japan, the mean BMI has increased in men, in whom the prevalence of obesity increased from 0.8% in 1976–80 to 2.01% in 1991–1995 (Yoshiike *et al.*, 2002). These rates are still markedly lower than the obesity rates in other developed countries. In contrast, a decreasing trend has been observed in Japan among women younger than 50 years of age, whereas an increasing trend has been observed among women older than 60 years of age (Yoshiike *et al.*, 2002).

Overweight and obesity are risk factors for a variety of chronic diseases, including diabetes mellitus, hypertension, cardiovascular diseases, and several types of cancer, including colorectal cancer (World Health Organization, 2003; World Cancer Research Fund and American Institute for Cancer Research, 2007). The effect of obesity on the risk of colorectal cancer can be attributed to three hormonal systems, namely, the insulin and insulin-like growth factor (IGF) axis, sex steroid hormones, and adipokines (Calle and Kaaks, 2004). Indeed, several epidemiologic studies have investigated the associations of these obesity-related biomarkers with the risks of colorectal cancer and of adenoma, a well-established precursor lesion for colorectal cancer (Otani *et al.*, 2006; Giovannucci, 2007; Le Marchand *et al.*, 2010; Ognjanovic *et al.*, 2010; Rinaldi *et al.*, 2010; Yamaji *et al.*, 2010, 2011).

In the first half of the 20th century, the incidence of colorectal cancer was very low in Japan. Since then,

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however, both incidence and mortality rates increased linearly up until the early 1990 s, and currently, Japan has some of the highest rates for this cancer in the world (Kono, 2004; Minami *et al.*, 2006; Center *et al.*, 2009). This major increase is most likely the result of lifestyle changes associated with the 'westernization' that occurred in Japan, such as an increased intake of meat, animal fat and alcohol, and obesity (Kono, 2004; Minami *et al.*, 2006; Center *et al.*, 2009). This susceptibility to the effect of the western lifestyle is supported by studies in Japanese migrants to the USA that documented a marked increase in the incidence of colorectal cancer as early as in the first generation of migrants, with rates that exceeded those of Caucasians (Shimizu *et al.*, 1987). Japanese American men in Los Angeles were subsequently shown to have an incidence rate similar to that in the Japanese in Japan in 2002 (Ferlay *et al.*, 2010). Another example of a Japanese migrant population is the Japanese in Brazil. Interestingly, however, colorectal cancer rates available for 1969–1979 suggest that the incidence had not increased among first-generation Japanese migrants to São Paulo, despite a high intake of red meat and higher BMI compared with Japanese in Japan (Tsugane *et al.*, 1990, 1994, 1996). Meanwhile, more recent data from 2000 showed that mortality from colorectal cancer among first-generation Japanese migrants to São Paulo had increased and become similar to that of Japanese in Japan (Iwasaki *et al.*, 2004, 2008).

These descriptive epidemiological findings suggest that Japanese in Japan, Brazil, and the USA are now at a similarly high risk for colorectal cancer. The prevalence of overweight and obesity, however, varies between these populations, with lower rates of obesity in Japan than in Japanese in Brazil and the USA (Tsugane *et al.*, 1994; Le Marchand *et al.*, 2010; Takachi *et al.*, 2011). Given that, apart from screening, differences in the prevalence of known risk factors determine most of the variation in the incidence of colorectal cancer across populations, the contribution of obesity to the risk of colorectal cancer in Japan might be relatively smaller than that of other risk factors. However, the impact of obesity on risk may differ between populations on the basis of body fat distribution. This is particularly true in Asia, considering that Asians have a greater percentage of body fat than western populations of the same age, sex, and BMI (WHO Expert Consultation, 2004). Therefore, comparison of obesity-related biomarkers between these three Japanese populations living in different countries might provide additional information on BMI to aid in understanding the high risk of colorectal cancer in Japan despite its relatively lean population. Comparison of these biomarkers may also provide a unique opportunity to further clarify their role in the etiology of this disease.

Here, we report a cross-sectional study that compared plasma levels of obesity-related biomarkers, namely, C-peptide, IGF-I, insulin-like growth factor-binding protein (IGFBP)-1, IGFBP-3, adiponectin, leptin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), C-reactive protein (CRP), total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides, between Japanese in Tokyo, Japanese Brazilians in São Paulo, and Japanese Americans in Hawaii.

Materials and methods
Study population
Japanese in Tokyo
Individuals included in the study were participants in the validation study of a semiquantitative food frequency questionnaire (FFQ) used for a case–control study of colorectal adenoma in Tokyo (Otani *et al.*, 2006; Yamaji *et al.*, 2009, 2010, 2011). They were selected from among examinees of the cancer screening program at the Research Center for Cancer Prevention and Screening, National Cancer Center, Japan, who fulfilled the following criteria: (a) age between 40 and 69 years; (b) residence in Tokyo and suburban prefectures; and (c) no previous or present diagnosis of cancer, cardiovascular disease, or diabetes mellitus. A total of 144 men and women provided weighed dietary records over four consecutive days, a self-administered FFQ, a fasting blood sample, from which serum and EDTA-2Na plasma was prepared and stored frozen at −80°C, and a 24-h urine sample between May 2007 and April 2008. The study design and data collection for the validation study have been described in detail elsewhere (Takachi *et al.*, 2011). The study was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan.

Japanese Brazilians in São Paulo
Individuals included in the study were participants of the validation study of a quantitative FFQ used for a case–control study of colorectal adenoma in São Paulo (Sharma *et al.*, 2009). They were selected from among participants in the case–control study of colorectal adenoma in São Paulo, and fulfilled the following criteria: (a) age between 40 and 79 years; (b) residence in the state of São Paulo for at least 6 months before recruitment; (c) at least three grandparents of pure Japanese ancestry; and (d) no history of colorectal cancer or history of other invasive cancer in the past 10 years. A total of 96 men and women provided food diaries over 4 consecutive days and a fasting blood sample between August 2008 and November 2009. Heparinized plasma was stored frozen at −80°C until analysis. The details of the validation study have been described elsewhere (Pakseresht *et al.*, 2012). The study was approved by the University of Hawaii Committee on Human Studies, as well as the Brazilian Ministries of Health, Science and Technology, and of Foreign Affairs, and the Brazilian National Ethics Commission.

Japanese Americans in Hawaii

Study participants from Hawaii were selected from among the Japanese American participants in an endoscopy-based case–control study of adenoma in Hawaii (Le Marchand *et al.*, 2010; Ognjanovic *et al.*, 2010). Seventy-eight participants aged 40–79 years who did not have a history of cancer completed a 4-day food record and provided a fasting blood sample between April 2002 and May 2007. Heparinized plasma was stored at − 80°C until analysis. The study was approved by the University of Hawaii Committee on Human Studies.

Laboratory analysis

Plasma samples from the three different studies were sent to the same laboratory, which measured all biomarkers. Plasma C-peptide level was measured by a chemiluminescent enzyme immunoassay method using a commercially available regent (Fujirebio, Tokyo, Japan) at SRL (Tokyo, Japan). Plasma IGF-I level was measured by a radioimmunoassay using a commercially available regent (Mitsubishi Chemical Medience, Tokyo, Japan). Plasma level of IGFBP-1 was measured by an enzyme immunoassay method using a commercially available kit manufactured by Mediagnost (Reutlingen, Germany). Plasma IGFBP-3 level was measured by immunoradiometric assay methods using a commercially available kit manufactured by Bioclone Australia Pty Ltd (Sydney, Australia). Assays of IGF-I, IGFBP-1, and IGFBP-3 were performed by a commercial laboratory (Mitsubishi Chemical Medience).

The following assays were performed with plasma and quality control samples immediately after thawing of samples stored at − 80°C. Duplicate analysis of each immunoassay was carried out on 96-well plates under yellow light to avoid sample and reagent degradation, and manufacturers' instructions were followed according to protocol. Human leptin and adiponectin were measured using kits from R&D Systems (Minneapolis, Minnesota, USA) (Catalog #DLP00 and #DRP300, respectively) from 1 : 10 diluted plasma. TNF-α and IL-6 were assayed from 100 μl plasma, each using two polystyrene-based ultrasensitive singleplex immunoassay kits (#LHC 3013 and #LHC 0063, respectively; Invitrogen, Camarillo, California, USA), and fluorescent intensities were obtained using a dual-laser-based fluorescent analyzer (Luminex 200; Luminex Corp., Austin, Texas, USA).

Plasma total cholesterol, HDL cholesterol, and triglycerides were analyzed from 9 μl of plasma using a clinical chemistry analyzer (Cobas MiraPlus; Roche Diagnostics, Indianapolis, Indiana, USA) and test kits on the basis of oxidase/peroxidase reactions (kits #C7510, #H7545 and #T7532, respectively; Pointe Scientific, Lincoln Park, Michigan, USA). LDL cholesterol was computed using the Friedwald equation: total cholesterol − HDL cholesterol − triglycerides/5 (Friedewald *et al.*, 1972).

CRP levels were measured from 24 μl of plasma using the Cobas analyzer and a latex particle-enhanced immunoturbidimetry-based kit from Pointe Scientific.

We used EDTA-2Na plasma samples from Japanese in Tokyo and heparinized plasma samples from Japanese Brazilians and Japanese Americans for the present study. To calibrate values from EDTA-2Na plasma samples, we measured all biomarkers above in both EDTA-2Na and heparinized plasma samples prepared from the same participants ($n = 15$). In addition, we included blind triplicate heparinized plasma samples from 15 participants in each assay as quality control. Lower detection limits (LODs) and intra-assay coefficients of variation (CV) for each biomarker are presented in Table S1. CVs were 5% or lower for C-peptide, IGF-I, IGFBP-1, IGFBP-3, total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides; between 10 and 20% for adiponectin, leptin, and CRP; and over 20% for TNF-α and IL-6.

Statistical analysis

We excluded participants whose plasma samples were not available, leaving 142 Japanese in Tokyo, 79 Japanese Brazilians in São Paulo, and 78 Japanese Americans in Hawaii for inclusion in the present analyses.

Measurement values below the LOD were assigned half the value of the LOD if measurable values below the LOD were not available. For each biomarker, measurement values from heparinized plasma samples were linearly regressed on those from EDTA-2Na plasma samples among 15 participants. The intercepts and slopes of these regressions were then used to estimate calibrated values for Japanese in Tokyo on the basis of measurement values from EDTA-2Na plasma samples. All values were natural log-transformed to produce approximately normal distributions, except for total, LDL, and HDL cholesterol. We excluded outliers, which were defined as values below or above the value equal to three times the interquartile range on the basis of the overall study population (Table S1). Pearson correlation coefficients between BMI and obesity-related biomarkers were calculated for the three populations separately. Geometric or arithmetic mean levels and their 95% confidence intervals were calculated for each of the three populations using multivariate regression analysis with adjustment for age (continuous), sex, season (spring, summer, autumn, winter), fasting status (< 10 and > 10 h), and BMI (continuous). Although participants were asked to provide fasting blood samples, 71 participants (66 in Tokyo, three in São Paulo, and two in Hawaii) provided samples within 10 h after their last meal. We therefore adjusted for fasting status in the multivariate regression analysis. Differences in the mean levels between the three populations were tested by analysis of covariance. Because more than half of the participants had plasma levels of TNF-α and IL-6 below the LOD, adjusted odds ratios and 95% confidence intervals were calculated using

an unconditional logistic regression model to compare the proportion of samples with detectable levels between the three populations. For comparisons between populations, each population was successively used as the reference group. All reported P values are two-sided, and significance level was set at P less than 0.05. All statistical analyses were carried out using SAS software version 9.1 (SAS Institute, Inc., Cary, North Carolina, USA).

Results
Table S1 shows the numbers of participants with values below the LOD and with an insufficient amount of plasma for each assay. The numbers of participants whose values were below the LOD were 140 for IL-6, 122 for TNF-α, 48 for CRP, 12 for leptin, and 5 or fewer for the other biomarkers. The distribution of samples below the LOD between the three populations differed significantly for TNF-α and CRP, but not for IL-6 and leptin (data not shown in Table). Further, no significant differences in demographic and anthropometric factors or the biomarkers listed in Table 2, except for TNF-α, IL-6, and CRP, were observed between participants with values below and over the LOD for TNF-α and IL-6 (data not shown in Table). About 50–60 participants were excluded for TNF-α and IL-6 and about 10 or fewer participants were excluded for the other biomarkers because of an insufficient amount of plasma. Finally, the number of eligible participants for the present analyses varied between 239 and 299.

Adjusted means for age and anthropometric factors in the three populations are compared in Table 1. Mean age was significantly lower for Japanese in Tokyo than for Japanese Brazilians in São Paulo and Japanese Americans in Hawaii. The proportion of men was 48% for Japanese in Tokyo, 35% for Japanese Brazilians, and 47% for Japanese Americans (data not shown). The mean BMI was significantly different between populations, with Japanese in Tokyo having the lowest mean BMI, followed by Japanese Brazilians, and Japanese Americans. Mean height was lower in Japanese Brazilians than in the other two populations.

Table S2 shows Pearson correlation coefficients for BMI and obesity-related biomarkers among the three populations. The C-peptide level was associated positively with leptin and CRP level and associated inversely with adiponectin level among the three populations. IGF-I level was associated positively with the IGFBP-3 level and associated inversely with IGFBP-1 among the three populations. Similarly, the IGFBP-1 level was associated positively with the adiponectin level and associated inversely with leptin among the three populations.

Table 2 shows a comparison of obesity-related biomarkers in plasma among Japanese in Tokyo, Japanese Brazilians in São Paulo, and Japanese Americans in Hawaii. Plasma levels of C-peptide and leptin were significantly different between the populations, with Japanese in Tokyo having the lowest mean, followed by Japanese Brazilians and Japanese Americans, although the difference was of borderline significance for C-peptide between Japanese in Tokyo and Japanese Brazilians. The mean adiponectin was higher in Japanese in Tokyo than in the other two populations. Stratified analyses by BMI categories (under and above 25 kg/m^2) showed the same statistically significant difference between Japanese in Tokyo and Japanese Americans irrespective of BMI, except for adiponectin for the category of BMI over 25 (Table 3).

Proportions of detectable samples for TNF-α and IL-6 tended to be lower in Japanese Americans than in the other two populations (Table 2). A statistically significant difference in TNF-α was observed between Japanese Brazilians and Japanese Americans, but no significant difference was found for IL-6. Plasma CRP levels did not differ statistically between the populations. Stratified analyses by BMI categories (under and above 25 kg/m^2) showed similar findings irrespective of BMI category (Table 3).

Plasma IGF-I levels differed significantly between populations, with Japanese in Tokyo having the lowest mean, followed by Japanese Brazilians and Japanese Americans. However, the difference was only significant between Japanese in Tokyo and Japanese Americans. Plasma IGFBP-3 levels were significantly higher in Japanese in Tokyo, whereas plasma IGFBP-1 levels were significantly higher in Japanese Brazilians than in the other two populations. In the stratified analyses by BMI category (under and above 25 kg/m^2), plasma IGF-I levels differed significantly between populations in the category of BMI under 25, but not in the category of BMI over 25 (Table 3), although the difference was only significant between Japanese in Tokyo and Japanese Brazilians in the category of BMI under 25. Similar results were obtained for IGFBP-1 in the category of BMI over 25. A significant difference in the mean IGFBP-3 level was observed between Japanese in Tokyo and Japanese Americans irrespective of BMI category.

Plasma levels of total cholesterol and LDL cholesterol were significantly lower in Japanese Americans than in the other two populations (Table 2). Plasma HDL cholesterol level was significantly higher whereas plasma triglycerides were significantly lower in Japanese in Tokyo than in the other two populations (Table 2). Stratified analyses by BMI category (under and above 25 kg/m^2) showed similar findings for total cholesterol and LDL cholesterol, irrespective of BMI category (Table 3). Significantly higher levels of HDL cholesterol and lower levels of triglycerides were observed in Japanese in Tokyo than in the other two populations in the category of BMI under 25, but not in the category of BMI over 25.

Table 1 Adjusted mean and 95% confidence interval for demographic and anthropometric factors in Japanese populations in Tokyo, Japan, in São Paulo, Brazil, and in Hawaii, USA

	Tokyo	São Paulo	Hawaii	P for difference		
				P^a	P^b	P^c
Age						
Number of participants	142	79	78	0.01	0.01	0.89
Multivariate[d]	58.6	62.1	61.9			
95% CI	57.1–60.1	60.1–64.2	59.9–64.0			
BMI (kg/m^2)						
Number of participants	142	79	78	< 0.01	< 0.01	< 0.01
Multivariate[e]	22.2	24.8	26.9			
95% CI	21.7–22.8	24.0–25.6	26.1–27.6			
Height (cm)						
Number of participants	142	79	78	< 0.01	0.52	0.04
Multivariate[e]	161.5	159.1	161.0			
95% CI	160.5–162.4	157.9–160.4	159.7–162.2			
Waist circumference (cm)						
Number of participants	41	79	78	< 0.01	< 0.01	0.04
Multivariate[e]	77.4	87.0	90.2			
95% CI	74.5–80.3	84.9–89.1	88.1–92.3			
Hip circumference (cm)						
Number of participants	41	79	78	0.67	< 0.01	< 0.01
Multivariate[e]	94.8	95.5	101.8			
95% CI	92.2–97.3	93.6–97.3	100.0–103.6			

CI, confidence interval.
[a]P values for testing differences in the mean levels between Japanese in Tokyo and Japanese Brazilians in São Paulo.
[b]P values for testing differences in the mean levels between Japanese in Tokyo and Japanese Americans in Hawaii.
[c]P values for testing differences in the mean levels between Japanese Brazilians in São Paulo and Japanese Americans in Hawaii.
[d]Adjusted for sex.
[e]Adjusted for sex and age.

Discussion

In this cross-sectional study, we found significantly lower levels of C-peptide (a marker of insulin secretion) and IGF-I in Japanese in Tokyo than in Japanese Americans, and lower levels of leptin and triglycerides and higher levels of adiponectin, IGFBP-3, and HDL cholesterol in Japanese in Tokyo than in the other two populations. In contrast, we found significantly higher levels of C-peptide and leptin in Japanese Americans than in the other two populations. We also observed significantly higher levels of IGF-I and triglycerides, and lower levels of adiponectin, IGFBP-3, and HDL cholesterol in Japanese Americans than in Japanese in Tokyo, but no significant difference in these markers was found between Japanese Americans and Japanese Brazilians. In stratified analysis by BMI, similar results were observed in the category of BMI under 25 kg/m^2. As our comparisons were made by adjusting for BMI, our findings indicate that the differences in these biomarkers might be explained not by BMI, but by other determinants. This profile of obesity-related biomarkers, however, is in agreement with the finding of the lowest mean BMI of Japanese in Tokyo and the highest in Japanese Americans among the three Japanese populations studied. Although not all biomarkers have as yet been established as risk factors for colorectal cancer, these biomarker data, at least those for C-peptide and IGF-I (Chen *et al.*, 2013; Chi *et al.*, 2013), would be consistent with a lower risk for Japanese in Tokyo and a higher risk for Japanese Americans. Our findings therefore do not explain why Japanese in Japan have a high risk of colorectal cancer despite being lean. However, considering the difference in the distribution of BMI among the three populations, the contribution of obesity to their increased risk may be relatively small compared with the other risk factors. In contrast, the contribution of obesity to the increased risk in Japanese Americans may be relatively large. For example, alcohol consumption was 122 g/week among the control group in the case–control study of colorectal adenoma in Tokyo (Otani *et al.*, 2006), whereas it was 1.3 g/day among the control group in the case–control study of colorectal adenoma in Hawaii, despite the fact that the sample also included Whites and Hawaiians, in addition to Japanese (Le Marchand *et al.*, 2010). Similarly, the prevalence of nonsteroidal anti-inflammatory drug use was 7.5% for the control group in Tokyo and 46.8% for the control group in Hawaii (Otani *et al.*, 2006; Ognjanovic *et al.*, 2010).

We found significantly lower levels of C-peptide and leptin in Japanese Brazilians than in Japanese Americans and a higher IGFBP-1 level in Japanese Brazilians than in the other two populations. However, we observed a significantly higher triglyceride level and lower levels of adiponectin, IGFBP-3, and HDL cholesterol in Japanese Brazilians than in Japanese in Tokyo, but no significant difference in these markers between Japanese Brazilians and Japanese Americans. Our findings, at least for C-peptide, suggest a lower risk profile for colorectal cancer in Japanese Brazilians than in Japanese Americans.

We did not observe a clear difference in the TNF-α level or statistically significant differences in CRP and IL-6

Table 2   Adjusted geometric mean and 95% confidence interval for obesity-related biomarkers in three populations

	Tokyo	São Paulo	Hawaii	P for difference		
				P^a	P^b	P^c
C-peptide (ng/ml)						
Number of participants	140	79	78	0.07	< 0.01	< 0.01
Multivariate[d]	1.58	1.78	2.30			
95% CI	1.48–1.69	1.60–1.98	2.07–2.56			
Insulin-like growth factor-I (IGF-I) (ng/ml)						
Number of participants	142	79	78	0.09	< 0.01	0.22
Multivariate[d]	140.5	155.5	167.3			
95% CI	132.2–149.4	140.7–171.8	151.8–184.4			
Insulin-like growth factor-binding protein-3 (IGFBP-3) (µg/ml)						
Number of participants	141	76	77	< 0.01	< 0.01	0.08
Multivariate[d]	3.41	2.84	3.02			
95% CI	3.30–3.53	2.69–3.01	2.86–3.18			
Insulin-like growth factor-binding protein-1 (IGFBP-1) (ng/ml)						
Number of participants	135	78	78	< 0.0	0.55	< 0.01
Multivariate[d]	4.82	8.01	5.25			
95% CI	4.16–5.59	6.32–10.16	4.17–6.60			
Adiponectin (µg/ml)						
Number of participants	142	79	78	0.02	< 0.01	0.15
Multivariate[d]	7.80	6.22	5.38			
95% CI	7.04–8.65	5.26–7.36	4.57–6.34			
Leptin (ng/ml)						
Number of participants	142	79	78	< 0.01	< 0.01	0.01
Multivariate[d]	2.45	4.33	5.97			
95% CI	2.15–2.80	3.49–5.38	4.83–7.37			
Tumor necrosis factor-α (TNF-α) (pg/ml)[e]						
Number of participants	128	60	53			
Number of detected samples (%)	66 (51.6)	36 (60.0)	17 (32.1)			
Multivariate odds ratio (OR)[d]	1.00	1.13	0.48			
95% CI	Reference	0.51–2.55	0.21–1.13			
Multivariate OR[d]	0.88	1.00	0.43			
95% CI	0.39–1.98	Reference	0.19–0.98			
Multivariate OR[d]	2.07	2.35	1.00			
95% CI	0.89–4.84	1.02–5.42	Reference			
Interleukin-6 (IL-6) (pg/ml)[e]						
Number of participants	132	62	54			
Number of detected samples (%)	62 (47.0)	29 (46.8)	17 (31.5)			
Multivariate OR[d]	1.00	1.05	0.54			
95% CI	Reference	0.47–2.32	0.23–1.26			
Multivariate OR[d]	0.96	1.00	0.52			
95% CI	0.43–2.12	Reference	0.22–1.19			
Multivariate OR[d]	1.85	1.94	1.00			
95% CI	0.79–4.33	0.84–4.47	Reference			
C-reactive protein (mg/l)						
Number of participants	142	79	67	0.14	0.21	0.87
Multivariate[d]	0.32	0.48	0.46			
95% CI	0.25–0.43	0.31–0.76	0.29–0.73			
Total cholesterol (mg/dl)[f]						
Number of participants	142	79	67	0.35	< 0.01	< 0.01
Multivariate[d]	199.5	194.8	164.5			
95% CI	194.3–204.6	186.2–203.3	155.9–173.1			
Low-density lipoprotein (LDL) cholesterol (mg/dl)[f]						
Number of participants	142	79	67	0.27	< 0.01	< 0.01
Multivariate[d]	127.8	122.1	94.8			
95% CI	122.5–133.1	113.2–130.9	85.8–103.7			
High-density lipoprotein (HDL) cholesterol (mg/dl)[f]						
Number of participants	142	79	67	0.02	< 0.01	0.35
Multivariate[d]	54.5	48.9	46.5			
95% CI	52.0–57.1	44.7–53.1	42.2–50.7			
Triglycerides (mg/dl)						
Number of participants	142	79	67	< 0.01	0.01	0.25
Multivariate[d]	75.3	106.9	96.6			
95% CI	69.1–82.1	92.6–123.5	83.5–111.8			

CI, confidence interval.

[a]P values for testing differences in the mean levels between Japanese in Tokyo and Japanese Brazilians in São Paulo.

[b]P values for testing differences in the mean levels between Japanese in Tokyo and Japanese Americans in Hawaii.

[c]P values for testing differences in the mean levels between Japanese Brazilians in São Paulo and Japanese Americans in Hawaii.

[d]Adjusted for sex, age, season, fasting status, and BMI.

[e]Adjusted OR was calculated to compare the proportion of detected samples between the three populations.

[f]Arithmetic means were calculated.

Table 3 Adjusted geometric mean and 95% confidence interval for obesity-related biomarkers in three populations according to BMI category

	BMI under 25						BMI over 25					
					P for difference						*P* for difference	
	Tokyo	São Paulo	Hawaii	P^a	P^b	P^c	Tokyo	São Paulo	Hawaii	P^a	P^b	P^c
C-peptide (ng/ml)												
Number of participants	123	45	30				17	34	48			
Multivariate[d]	1.40	1.73	1.86	<0.01	<0.01	0.39	1.97	1.89	2.91	0.78	<0.01	<0.01
95% CI	1.32–1.49	1.52–1.96	1.62–2.13				1.58–2.46	1.53–2.34	2.39–3.54			
Insulin-like growth factor-I (IGF-I) (ng/ml)												
Number of participants	124	45	30				18	34	48			
Multivariate[d]	141.2	171.6	163.2	<0.01	0.053	0.54	143.9	138.4	166.4	0.75	0.22	0.051
95% CI	133.1–149.7	151.8–193.9	142.8–186.5				119.3–173.7	115.3–166.2	140.9–196.6			
Insulin-like growth factor-binding protein-3 (IGFBP-3) (µg/ml)												
Number of participants	123	44	30				18	32	47			
Multivariate[d]	3.36	2.96	2.89	<0.01	<0.01	0.56	3.69	2.78	3.18	<0.01	0.0498	0.03
95% CI	3.27–3.46	2.79–3.14	2.71–3.08				3.28–4.16	2.47–3.13	2.86–3.54			
Insulin-like growth factor-binding protein-1 (IGFBP-1) (ng/ml)												
Number of participants	120	44	30				15	34	48			
Multivariate[d]	5.70	7.83	7.26	0.04	0.15	0.68	3.89	8.58	4.60	0.01	0.57	0.01
95% CI	4.98–6.52	5.93–10.33	5.38–9.79				2.37–6.40	5.50–13.39	3.03–6.98			
Adiponectin (µg/ml)												
Number of participants	124	45	30				18	34	48			
Multivariate[d]	9.27	6.87	5.92	0.01	<0.01	0.27	4.39	4.65	3.77	0.78	0.44	0.18
95% CI	8.41–10.22	5.61–8.42	4.74–7.38				3.22–5.99	3.44–6.30	2.86–4.97			
Leptin (ng/ml)												
Number of participants	124	45	30				18	34	48			
Multivariate[d]	1.70	3.22	4.37	<0.01	<0.01	0.13	5.29	7.35	9.77	0.10	<0.01	0.06
95% CI	1.47–1.97	2.39–4.35	3.15–6.06				3.91–7.16	5.47–9.86	7.48–12.78			
Tumor necrosis factor-α (TNF-α) (pg/ml)[e]												
Number of participants	111	33	23				17	27	30			
Number of detected samples (%)	57 (51.4)	20 (60.6)	7 (30.4)				9 (52.9)	16 (59.3)	10 (33.3)			
Multivariate odds ratio (OR)[d]	1.00	1.33	0.43				1.00	0.97	0.54			
95% CI	Reference	0.49–3.62	0.14–1.30				Reference	0.19–4.83	0.12–2.46			
Multivariate OR[d]	0.75	1.00	0.32				1.03	1.00	0.55			
95% CI	0.28–2.05	Reference	0.09–1.11				0.21–5.16	Reference	0.17–1.80			
Multivariate OR[d]	2.35	3.12	1.00				1.87	1.80	1.00			
95% CI	0.77–7.14	0.90–10.79	Reference				0.41–8.54	0.56–5.84	Reference			
Interleukin-6 (IL-6) (pg/ml)[e]												
Number of participants	115	35	23				17	27	31			
Number of detected samples (%)	53 (46.1)	16 (45.7)	4 (17.4)				9 (52.9)	13 (48.2)	13 (41.9)			
Multivariate OR[d]	1.00	1.07	0.28				1.00	0.94	0.73			
95% CI	Reference	0.41–2.83	0.08–1.00				Reference	0.20–4.34	0.17–3.03			
Multivariate OR[d]	0.93	1.00	0.26				1.07	1.00	0.78			
95% CI	0.35–2.46	Reference	0.07–1.03				0.23–4.95	Reference	0.24–2.49			
Multivariate OR[d]	3.57	3.82	1.00				1.38	1.29	1.00			
95% CI	1.00–12.78	0.98–15.00	Reference				0.33–5.76	0.40–4.14	Reference			
C-reactive protein (mg/l)												
Number of participants	124	45	27				18	34	40			
Multivariate[d]	0.23	0.43	0.36	0.06	0.22	0.64	0.95	0.80	0.93	0.75	0.97	0.71
95% CI	0.17–0.30	0.24–0.77	0.19–0.69				0.43–2.12	0.36–1.76	0.45–1.95			
Total cholesterol (mg/dl)[f]												
Number of participants	124	45	27				18	34	40			
Multivariate[d]	203.0	197.2	163.1	0.34	<0.01	<0.01	187.3	192.3	167.7	0.61	0.04	<0.01
95% CI	197.7–208.2	186.3–208.1	150.8–175.3				172.4–202.2	177.7–207.0	154.0–181.4			

Table 3 (continued)

	BMI under 25			P for difference			BMI over 25			P for difference		
	Tokyo	São Paulo	Hawaii	P^a	P^b	P^c	Tokyo	São Paulo	Hawaii	P^a	P^b	P^c
Low-density lipoprotein (LDL) cholesterol (mg/dl)[f]												
Number of participants	124	45	27				18	34	40			
Multivariate[d]	129.9	122.3	95.1	0.21	<0.01	<0.01	119.0	123.9	99.3	0.66	0.06	<0.01
95% CI	124.7–135.1	111.5–133.2	82.9–107.3				102.4–135.7	107.6–140.2	84.0–114.5			
High-density lipoprotein (HDL) cholesterol (mg/dl)[f]												
Number of participants	124	45	27				18	34	40			
Multivariate[d]	58.5	52.4	47.4	0.049	<0.01	0.18	43.1	41.7	40.5	0.75	0.52	0.70
95% CI	55.8–61.2	46.8–58.0	41.1–53.6				36.6–49.7	35.3–48.2	34.5–46.5			
Triglycerides (mg/dl)												
Number of participants	124	45	27				18	34	40			
Multivariate[d]	67.0	103.2	89.5	<0.01	<0.01	0.19	104.7	117.7	109.2	0.58	0.83	0.64
95% CI	62.1–72.3	88.0–121.1	74.9–107.1				76.3–143.7	86.3–160.5	81.7–146.0			

CI, confidence interval.
[a]P values for testing differences in the mean levels between Japanese in Tokyo and Japanese Brazilians in São Paulo.
[b]P values for testing differences in the mean levels between Japanese in Tokyo and Japanese Americans in Hawaii.
[c]P values for testing differences in the mean levels between Japanese Brazilians in São Paulo and Japanese Americans in Hawaii.
[d]Adjusted for sex, age, season, fasting status, and BMI.
[e]Adjusted OR was calculated to compare the proportion of detected samples between the three populations.
[f]Arithmetic means were calculated.

levels between populations. This may be partly explained by the fact that levels of TNF-α and IL-6 were below the LOD in ∼50–60% of participants, and that the CV for TNF-α and IL-6 were over 20%.

We found significantly higher plasma HDL cholesterol levels and lower plasma triglycerides levels in Japanese in Tokyo than in the other two populations, as mentioned above. These findings are consistent with two previous reports (Tsugane et al., 1994; Schwingel et al., 2007). However, we did not observe significant differences in total cholesterol or LDL cholesterol between Japanese in Tokyo and Japanese Brazilians. Schwingel et al. (2007) also reported a similar finding for total cholesterol, but our previous study showed higher total cholesterol levels among Japanese Brazilian men than Japanese men (Tsugane et al., 1994). Interestingly, plasma levels of total cholesterol and LDL cholesterol were significantly lower in Japanese Americans in our study than in the other two populations. This contrasts with previous studies showing higher rates for coronary heart disease among Japanese men in Hawaii and California than in those in Japan (Robertson et al., 1977). This discrepancy might reflect the high prevalence of individuals treated with cholesterol-lowering medication in the USA (Roth et al., 2011), a variable that we did not collect.

Several methodological limitations of this study should be considered. Our findings might have been affected by differences in study methodology in the three populations. One major difference is the method of blood collection. We used EDTA-2Na plasma samples from Japanese in Tokyo, but heparinized plasma samples from Japanese Brazilians and Japanese Americans. Although both were plasma samples, differences in anticoagulants may have affected analytical values. To minimize this possibility, we measured all biomarkers in both EDTA-2Na and heparinized plasma samples in a subset of participants (n=15) and calibrated the values in the study using EDTA-2Na plasma samples. The difference in anticoagulants is therefore unlikely to have created the difference observed in analytes across the three populations. However, although the blood collection methods were somewhat different, we measured each biomarker in the same laboratory and batched samples so that the same numbers of samples from the three populations were analyzed in each analytical batch, minimizing the effect of laboratory variation. Another major source of variation may have been the way in which the participants were recruited at each study site. As Japanese in Tokyo were examinees of a cancer screening program, they were asymptomatic and may have been more health conscious. However, the Japanese Brazilians and Japanese Americans were generally symptomatic patients who underwent colonoscopy in each hospital. However, this difference is unlikely to have affected the findings of the study because several biomarkers differed significantly between the Japanese Americans and Japanese

Brazilians, but not between the Japanese in Tokyo and Japanese Brazilians.

In conclusion, we found significant differences in obesity-related biomarkers between the three Japanese populations. Our findings suggest that a risk profile for colorectal cancer predicted on the basis of these biomarkers would be most favorable in Japanese from Tokyo, followed by Japanese Brazilians and Japanese Americans.

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Conflicts of interest

There are no conflicts of interest.

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