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## Relation of dietary and lifestyle traits to difference in serum leptin of Japanese in Japan and Hawaii: The INTERLIPID Study

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### Abstract

**Background and Aims**—Previously, we found significantly higher serum leptin in Japanese-Americans in Hawaii than Japanese in Japan. We investigated whether differences in dietary and other lifestyle factors explain higher serum leptin concentrations in Japanese living a Western lifestyle in Hawaii compared with Japanese in Japan.

**Methods and Results**—Serum leptin and nutrient intakes were examined by standardized methods in men and women ages 40 to 59 years from two population samples, one Japanese-American in Hawaii (88 men, 94 women), the other Japanese in central Japan (123 men, 111 women). Multiple linear regression models were used to assess role of dietary and other lifestyle traits in accounting for serum leptin difference between Hawaii and Japan. Mean leptin was significantly higher in Hawaii than Japan ( $7.2 \pm 6.8$  vs  $3.7 \pm 2.3$  ng/ml in men,  $P < 0.0001$ ;  $12.8 \pm 6.6$  vs  $8.5 \pm 5.0$  in women  $< 0.0001$ ). In men, higher BMI in Hawaii explained over 90% of the difference in serum leptin; in women, only 47%. In multiple linear regression analyses in women, further adjustment for physical activity and dietary factors - - alcohol, dietary fiber, iron - - produced a further reduction in the coefficient for the difference, total reduction 70.7%;  $P$  value for the Hawaii-Japan difference became 0.126.

**Conclusion**—The significantly higher mean leptin concentration in Hawaii than Japan may be attributable largely to differences in BMI. Differences in nutrient intake in the two samples were associated with only modest relationship to the leptin difference.

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## Introduction

Leptin, named after the Greek *leptos* meaning thin, was identified by positional cloning of the mouse obese (*ob*) gene as a key molecule in the physiological regulation of body weight and energy balance [1]. Leptin is produced and secreted mainly by adipocytes. It acts on the hypothalamus, altering energy intake by decreasing appetite and increasing energy expenditure via sympathetic stimulation of several tissues [2]. Obese individuals, however, remain hyperphagic despite their high circulating leptin concentrations, indicating hypothalamic insensitivity to leptin [3]. Despite hypothalamic leptin resistance, the sympathoexcitatory effect of leptin is preserved after either systemic or central neural administration of leptin [4]. These findings led to the concept of selective leptin resistance [5].

INTERLIPID, an ancillary study of the International Study of Macro/micronutrients and Blood Pressure (INTERMAP), investigated coronary heart disease (CHD) risk factors in four Japanese population samples in Japan and a Japanese–American population sample in Hawaii [6,7]. In INTERMAP, dietary surveys were conducted with a highly standardized protocol in 17 random population samples in four countries (Japan, People's Republic of China, the U.K., and the U.S.) [8].

Although there have been several studies on the association between dietary intakes and leptin concentrations, discrepancies are found as to the effects of alcohol and macronutrients on leptin. [16-21]. Since our samples included Japanese in Japan and Japanese-Americans; an ethnically homogenous cohort with a wide range of BMIs (from 17.2 to 47.0 kg/m<sup>2</sup>) due to differences in lifestyle related factors, we thought we might be able to solve the problems on the relationship between dietary factors and serum leptin concentration. Furthermore, in INTERLIPID data analyses, we found for both genders significantly higher serum leptin concentrations of middle-aged Hawaiian Japanese-Americans than Japanese living in Japan. We hypothesized that these differences are largely related to differences in dietary and non-dietary lifestyle factors between the two population samples; data from previous studies show that Japanese-Americans in Hawaii and Japanese in Japan have significantly different dietary patterns as well as body mass index (BMI) [6-8].

## Methods

Detailed methods of the INTERMAP Study have been described [8]. They are summarized here. Two standardized blood pressure measurements were made on each participant on four different days; medical and lifestyle data were collected with standardized forms; four in-depth multi-pass 24-h dietary recalls and two timed 24-h urine collections were obtained. In addition, non-fasting blood was drawn from INTERLIPID participants [6,7]. We used data on analytes measured in these samples, as well as INTERMAP dietary and other data.

## Participants

INTERLIPID participants ages 40–59 years were from five INTERMAP research centers: four in Japan and one in Hawaii [6,7]. For the present study, serum leptin concentrations were measured in two samples, one from Japan and one from Hawaii. The two populations samples were: (1) Japanese residents in Aito Town, a rural town in Shiga prefecture, central Japan (129 men and 129 women) and (2) third and fourth generation offspring of Japanese emigrants living in Honolulu, Hawaii (100 men and 106 women). Participants in Honolulu were asked about the ethnicity of their mother and father; those included in the study responded 100% Japanese to both. Aito Town was chosen because it was the only Japanese community sample; the other three samples were of factory workers. There were only small differences in lifestyle and dietary habits among the four samples in Japan; differences of

those variables between Japan and Hawaii samples were larger [8]. Among those in these two samples, 48 persons (24 Japanese, 24 Japanese-American) were excluded because volume of their stored serum specimen was not enough to measure leptin, leaving 234 Japanese individuals (123 men and 111 women) and 182 Japanese-Americans (88 men and 94 women).

Ethics committees of the Shiga University of Medical Science, the Pacific Health Research Institute, and Northwestern University approved the study protocol. Written informed consent was obtained from all participants.

### **Anthropometric and lifestyle assessment**

Participants visited the research centers four times on two pairs of consecutive days three weeks apart on average. Height and weight with light clothes were measured at each visit. Using a questionnaire, trained certified observers inquired about physical activity, smoking status, previous medical history of cerebro-cardiovascular diseases/diabetes, use of medication, et al. Hypertension was defined as systolic blood pressure  $\geq 140$  mmHg, diastolic blood pressure  $\geq 90$  mmHg, or use of anti-hypertensive medication. Diabetes mellitus was defined as HbA1c  $\geq 6.5\%$ , or use of anti-diabetic medication.

### **Dietary assessment**

Four in-depth multi-pass 24-h dietary recalls per participant were conducted during the four visits by specially trained dietary interviewers. Prior to data collection, a supervising nutritionist in each country trained all interviewers and certified that they had the appropriate skills to conduct dietary interviews and process dietary data using computers. Standardized on-going quality control procedures were adopted to optimize quality of dietary data throughout data collection [8]. All participants for the present study attended all 4 study visits; their energy intakes from all 24-hour dietary recalls were between 500 and 5000 kcal/day.

### **Biochemical measurements**

For the INTERLIPID Study, non-fasting blood was drawn on the second day of the first two-day visit pair. Serum and plasma were obtained by centrifugation within 30 min of blood drawing and immediately refrigerated. Within 24-hours, all specimens were frozen and stored locally at  $-70^{\circ}\text{C}$ . Samples from the Hawaiian and Japanese centers were shipped to a central laboratory in Japan on dry ice. Individual samples from the two centers were randomly allocated for analysis to avoid systematic measurement bias. The central laboratory was standardized by the Lipid Standardization Program, Centers for Disease Control and Prevention, Atlanta, GA, U.S.A.; it successfully met the criteria of precision and accuracy of control [9]. The laboratory is currently a member of the Cholesterol Reference Method Laboratory Network (CRMLN) [10]. Serum concentrations of total cholesterol (TCH), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were directly measured by enzymatic methods on an auto-analyzer (Hitachi 7107, Tokyo, Japan). Serum leptin concentrations were measured by immunoassays from Linco Research [Millipore (Billerica, MA)]. We included standard sera of known values with each batch; there were no significant differences in the standard serum concentrations among the batches. The analytical coefficients of variation (CV) were less than 3% for TCH, LDL-C, HDL-C, and triglycerides. Although significant postprandial increase in blood triglyceride concentrations occurs, postprandial stability of TCH, HDL-C, and LDL-C have been demonstrated [11]. Postprandial stability of leptin also has been shown in normal and obese persons, as well as in patients with type II diabetes mellitus [12]

## Data analyses

For each person, means of the individual nutrients from the four 24-h dietary recalls were used in the analyses. Data are presented as the contribution to total energy intake (% kcal) from total available carbohydrates, total fat, monounsaturated fatty acids (MUFA), saturated (SFA), polyunsaturated (PUFA), long chain n-3 PUFAs,  $\alpha$ -linolenic acid, n-6 PUFA, linoleic acid, dietary cholesterol (mg/1000 kcal), total dietary fiber (g/1000kcal), magnesium (mg/1000 kcal), iron (mg/1000 kcal), phosphorus (mg/1000 kcal), and alcohol (% kcal). In INTERMAP, intakes of arachidonic acid (ARA, n20:4), eicosapentaenoic acid (EPA, n20:5), docosahexaenoic (DHA, n22:6), and docosapentaenoic acid (DPA, n22:5) were estimated. ARA intake was included in the analyses because of its reported adverse (including atherogenic) effects. Intakes of EPA, DHA, and DPA were included since fish intake was sizably higher among Japanese in Japan than among Japanese-Americans in Hawaii. EPA, DHA, and DPA intakes of individuals were highly intercorrelated (Pearson partial correlation coefficients between any two of the three fatty acids greater than 0.9). Keys dietary lipid score, predictive of serum total cholesterol, was calculated as  $1.35 \times (2\text{SFA} - \text{PUFA}) + 1.5 \times \text{C}^{1/2}$ , where SFA is the percentage of total kilocalories from saturated fatty acids; PUFA, percentage from polyunsaturated fatty acids; C dietary cholesterol in mg/1000 kcal [13]. BMI was calculated as weight divided by height squared ( $\text{kg}/\text{m}^2$ ). Sample average number of cigarettes smoked per day was calculated for current smokers only and for all persons including non-smokers. Student's t-tests were used to compare means of Japanese and Japanese-Americans; chi-squared tests, to compare smoking rates, use of lipid lowering and anti-hypertensive drugs.

Based on significant Hawaii-Japan univariate differences for non-dietary and dietary variables in both men and women, gender-specific multiple linear regression models were used to examine the relations of dietary factors and physical activity to Hawaii-Japan differences in leptin concentration, with control for non-dietary variables [14]. Because the distribution of serum leptin was positively skewed, a logarithmic transformation was used to normalize the distribution (log-leptin). The basic model (Model 1) included age and an indicator for site (Hawaii=1, Japan=0) to obtain the gender-specific age-adjusted coefficient for Hawaii-Japan differences in log-leptin. Addition of number of cigarettes smoked per day or physical activity, BMI, or postprandial hour to Model 1 was also examined. Model 2 included physical activity in addition to Model 1 variables. Model 3 included BMI and number of cigarettes smoked per day in addition to Model 2 variables. Then, each dietary factor possibly explaining remaining Hawaii-Japan differences in leptin concentration was added to Model 2 and 3 separately, and percentage total and further reduction in the site coefficient was calculated to assess influence of the added variable on Hawaii-Japan log-leptin differences. Finally, dietary variables were included in combinations to assess their joint impact on Hawaii-Japan differences in log-leptin.

## Results

### Demographic Characteristics and Blood Chemistry Concentrations

For both men and women, mean leptin concentration was significantly higher in Hawaii than in Japan (Table 1). For both participants in Hawaii and Japan, mean leptin concentration was significantly higher in women than in men (both  $P < 0.0001$  by Student's t-test on log-leptin). Mean BMI was significantly higher in men than in women in Hawaii ( $P = 0.0001$ ), however, it was not different between men and women in Japan ( $P = 0.97$ ). Average height was similar for Japanese in Japan and Japanese in Hawaii. Average BMI was significantly higher in Hawaii than in Japan ( $P < 0.0001$  and  $0.0001$ ) for both men and women with the difference greater for men than women. Prevalence of premenopause was not different between women in Japan and Hawaii ( $P = 0.23$ ). Smoking rate in men and mean for moderate + heavy

physical activity (hours/day) in men and women were lower in Hawaii than in Japan ( $P < 0.0001$ ). Antihypertensive and lipid lowering drugs were used by a significantly greater percentage of individuals of both genders in Hawaii than in Japan. Mean postprandial hour was not different between men in Japan and Hawaii, however it was significantly shorter in women in Japan than in Hawaii ( $P=0.04$ ). Mean LDL-C, HbA1c in men and women were higher in Hawaii than in Japan.

### Nutrient Intakes

Most displayed nutrient intakes were significantly different across the two samples (Table 2). Mean intakes (% kcal) of total fat, SFA, MUFA, PUFA, n-6 PUFA, and ARA in men and women were higher in Hawaii than in Japan. Participants in Hawaii consumed lower percentages of total calories from carbohydrates, n-3 PUFA, EPA, DHA, DPA (also less cholesterol) than in Japan ( $P<0.0001$ ). Mean Keys dietary lipid score in men was significantly higher in Hawaii than in Japan ( $P<0.0001$ ). Men in Hawaii ingested lower percentage of total calories from alcohol than men in Japan ( $P< 0.0001$ ).

### Relation of Dietary Variables to Leptin and Hawaii-Japan Leptin Differences

Table 3 gives coefficients from multiple linear regression models used to examine relations of factors to Hawaii-Japan difference in log-leptin concentration in men and women. In men, addition to Model 1 of BMI reduced this leptin difference by 92.4%. The P-value for the Honolulu-Aito Town difference in serum leptin decreased from  $<0.0001$  to 0.51. Addition of physical activity to Model 1 (Model 2) reduced Hawaii-Japan difference in log-leptin only slightly (13.3%); the P-value remained significant ( $<0.0001$ ). Because BMI had strong impact on Hawaii-Japan difference in log-leptin, inclusion of BMI in models may washout potential effects of nutrient factors on leptin. Thus, additions of nutrient factors to Model 2 (without BMI) were examined one by one. The results of nutrient factors with significant relationship with log-leptin in men or women are shown. In men, only dietary fiber had significant relationship with log-leptin (coefficient  $b=-0.020$ ,  $P=0.007$ ). Addition of BMI and number of cigarettes smoked per day to Model 2 (Model 3) reduced Hawaii-Japan difference in log-leptin remarkably (99.99%,  $P=0.99$ ). Thus in men, the sizable difference in BMI across the two populations statistically accounted for most of the serum leptin difference.

In women, addition to Model 1 of BMI reduced the coefficient for Hawaii-Japan leptin difference by 46.6%. Addition of postprandial hour to Model 1 did not change the coefficient for Hawaii-Japan leptin difference. Addition of physical activity to Model 1 (Model 2) reduced Hawaii-Japan difference in log-leptin only slightly (0.1%); the P-value remained significant ( $<0.0001$ ). By addition of dietary factors to Model 2, dietary factors found to have significant relationship with log-leptin were arachidonic acid ( $b=1.3$ ,  $P=0.004$ ), Keys dietary lipid score ( $b=0.0045$ ,  $P=0.03$ ), alcohol intake ( $b=-0.021$ ,  $P=0.03$ ), and dietary fiber ( $b=-0.012$ ,  $P=0.04$ ). With addition of BMI and number of cigarettes smoked per day to Model 2, percent reduction in the coefficient increased slightly to 49.0% (Model 3). Except for alcohol, addition to Model 3 of nutrients shown in Table 2, singly, had no significant independent relation to leptin, and these dietary variables singly did not reduce the coefficient for log-leptin difference significantly. With addition to Model 3 of alcohol (%kcal), overall percent reduction in the coefficient was 49.1%; alcohol had a significant independent inverse relation to log-transformed serum leptin ( $P=0.023$ ). The P-value for the Honolulu-Aito Town difference in log-leptin decreased slightly from  $<0.0001$  to 0.0006. With addition to Model 3 of alcohol, total dietary fiber and iron (mg/1000kcal), percent reduction in the coefficient increased to 70.7%. The P-value for the Honolulu-Aito Town difference in log-transformed serum leptin decreased from  $<0.0001$  to 0.126, although none of these variables except for alcohol, had a significant independent relation to leptin.

## Discussion

Main findings here were: serum leptin concentrations were significantly higher in population samples of Japanese-Americans in Hawaii than Japanese in Japan, people of the same genetic background. In men, adjustment for higher BMI in Hawaiian Japanese-Americans reduced the log-leptin Hawaii -Japan difference by 92%, to a nonsignificant level. In women, BMI reduced this difference by only 47%; further adjustment for three dietary factors (iron, fiber, alcohol) produced further reduction in the difference, to 71%.

There have been several studies on the association between dietary intakes and leptin concentrations. Raben et al. examined the acute effect of four isocaloric meals rich in alcohol, protein, carbohydrate or fat on serum leptin concentrations; they found leptin concentrations greatly suppressed the first 4 hours after the alcohol meal [15]. A very slight suppression of leptin concentration was seen the first 3 hours after the other meals. Postprandial stability of leptin also has been shown in normal and obese persons, as well as in patients with type II diabetes mellitus [12]. Therefore, slight difference in postprandial hour between women in Japan and Hawaii found in our study might not have contributed the site difference in leptin concentrations. In fact, addition of postprandial hour to Model 1 did not change the coefficient for Hawaii-Japan leptin difference in our study.

There have been discrepancies as to the long-term effect of alcohol on leptin; some reported an increase, others a decrease or no change [16-18]. A cross-sectional study by Yannakoula et al. in 120 healthy Greek male and female students found that protein, carbohydrate, and fat intakes were not correlated with leptin concentrations in multiple regression analyses [19]. Another cross-sectional study by Murakami et al. in 424 female students reported that only dietary fiber was inversely correlated with leptin concentrations independent of potential confounding factors, including BMI; protein, total fat, SFA, MUFA, PUFA, and carbohydrate were not correlated with leptin concentration [20]. A study by Chu et al. among 268 normal weight and overweight men, on the other hand, found that total fat and MUFA intakes were positively associated with leptin concentrations independent of BMI and other confounding factors only in men with BMI < 25.0 kg/m<sup>2</sup> [21].

Dietary fiber was the only nutrient that had consistently significant inverse relationship with leptin in both men and women in our study. Our results agree with those of Murakami et al. [20] High intake of dietary fiber has been linked to a lower risk of coronary heart disease and type 2 diabetes mellitus in several epidemiological studies [22-24]. Because dietary fiber is indigestible and not absorbed in the intestine, the benefit from intake of dietary fiber is thought that the viscosity of dietary components may modify the rate and the site of nutrient absorption, fermentation of polysaccharides in the large intestine may produce products that affect metabolism, and binding of compounds, such as bile acids, affects the excretion of the compounds [25]. Another possible mechanism may be a change in dietary pattern, resulting in a diet that is lower in saturated and trans-unsaturated fats and cholesterol and higher in protective nutrients such as an unsaturated fatty acids, minerals, folate, and antioxidant vitamins. It is understandable that these beneficial effects resulted in inverse relationship of dietary fiber with leptin. However, these relationships were not independent of BMI in our study.

Our results in women support the conclusion that alcohol intake suppresses leptin concentration by Raben et al. [15]. The relationship in our study, however, was not independent of BMI. We found direct association of arachidonic acid with leptin in women. No previous epidemiological study examined this relationship, Fain et al. found that arachidonic acid stimulated leptin release by explants of subcutaneous adipose tissue from obese humans [26]. This relation in our study also was not independent of BMI. BMI

dependent direct association of Keys dietary lipid score with leptin in our study in women is not found in previous studies, however, it is understandable.

As to other lifestyle factors possibly related to leptin concentration, several studies have examined the response of leptin to exercise. It appears that nonexhaustive exercise of short duration does not affect leptin, whereas bouts demanding high-energy decrease leptin concentration [27-29]. Sedentary lifestyles, as represented by average hours of television watching per week, have been found by Fung et al. to associate directly with leptin concentration [30]. Smoking was reported in some studies to reduce leptin concentration [31,32], however, one study found little relationship between smoking and leptin in Greek men [33]. In our regression models without BMI, in men, but not in women, there were modest inverse associations of smoking and physical exercise with leptin concentration.

Collectively, dietary factors that had significant association with leptin in our study were dietary fiber in both sexes, and alcohol in women; these were BMI dependent associations. Three major nutrient factors did not have significant associations with leptin. These results mostly agree with those of previous studies. However, BMI independent association of dietary fiber with leptin in the study by Murakami et al. [20] was not confirmed in our study. Very wide range of BMI in our participants, whereas a relatively narrow range of BMI in the study by Murakami et al. might have caused the slight difference.

Japanese living in Japan had higher intake of dietary cholesterol than those in Hawaii. Although, we have not food group data yet, previous studies reported that egg consumption contributed to 26 to 32% to total dietary cholesterol intake in the U.S. [34,35], whereas that in Japan was reported to contribute to about 48% in Japan [36]. Mean LDL concentration was higher in men and women in Hawaii than those in Japan despite a lower intake of dietary cholesterol was due mostly to a higher intake of SFA in persons in Hawaii than those in Japan.

The main strengths of the present study are: (1) use of population-based samples; (2) standardized collection of high-quality BP and nutrition data; (3) use of an improved nutrient database; and (4) use of multiple quality-control procedures. The study was limited by its two-sample cross-sectional design, and small number of participants. Findings may or may not be generalizable to all Japanese and to other populations. Second, despite high-quality dietary nutrient data acquisition methods, limitation in reliability may still bias against nutritional variables influence in multivariate models. Third, we did not measure waist circumference (WC). However, we showed previously in a population-based study that BMI and WC correlated very well in men and women, and that BMI could be used instead of WC in a study when the latter was not available [37]. Although the measurement of WC is widely advocated as a simple anthropometric marker of health risk, there remains no uniformly accepted protocol [38,39]. Fourth, we did not measure subcutaneous adipose tissue area and visceral adipose tissue area by computer tomography. There were more dietary factors in women than in men that were significantly associated with leptin. In addition, BMI contributed more strongly in men than in women for explanation of the log-leptin Hawaii-Japan difference. These sex specific difference in the association of BMI and dietary factors with leptin found in the present study may be related to sex related difference in fat mass distribution. Data on subcutaneous adipose tissue area and visceral adipose tissue area might have helped the interpretation.

*In conclusion*, Mean leptin concentration was significantly higher in Hawaii than in Japan for both genders. In men, this difference related overwhelmingly to BMI; in women, to BMI and physical activity, with three specific dietary factors (iron, fiber, alcohol) playing a modest role in further reduction of the leptin difference.

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Table 1

Demographic Characteristics and Blood Chemistry Concentrations, by Gender—INTERLIPID Study, Aito Town, Japan and Honolulu, Hawaii, USA, 1997-99

Variable	Men			Women		
	Japan	Hawaii	P	Japan	Hawaii	P
Number of People	123	88		111	94	
Age (years)	49.5±6.1	50.7±5.2	0.12	50.0±6.2	49.4±4.9	0.47
Height (m)	1.67±0.06	1.67±0.06	0.99	1.54±0.05	1.54±0.05	0.76
BMI (kg/m <sup>2</sup> )	23.3±2.7	28.4±4.3	<0.0001	23.3±2.9	25.6±5.1	0.0001
Pre-menopause (%)	-	-	-	40.5%	48.9%	0.23
Smokers (%)	53.0	10.2	<0.0001	0.9	4.8	0.09
Moderate or heavy physical activity (h/day)	4.9±4.7	2.2±2.9	<0.0001	5.0±4.5	1.2±1.7	<0.0001
Hypertension (%)	22.2	32.7	0.09	13.1	24.8	0.03
Diabetes (%)	2.4	7.1	0.10	0.9	3.7	0.16
HTN Rx (%)	5.7	27.3	<0.0001	9.3	24.5	0.003
Lipid Rx (%)	4.9	17.1	0.004	2.7	9.6	0.04
Postprandial hour (h)	3.5±2.4	4.2±4.2	0.26	2.9±2.0	3.8±3.8	0.04
Leptin* (ng/ml)	3.7±2.3	7.2±6.8	<0.0001	8.5±5.0	12.8±6.6	<0.0001
Total cholesterol (mg/dl)	203.0±30.0	212.0±28.5	0.09	204.5±30.3	210.0±32.3	0.22
HDL-C (mg/dl)	53.3±14.0	50.5±10.4	0.07	61.2±16.4	59.5±13.1	0.41
LDL-C (mg/dl)	125.3±27.7	135.2±28.4	0.01	124.9±29.2	135.7±33.0	0.014
HbA1c (%)	4.7±0.6	5.0±0.8	0.0006	4.5±0.4	4.8±0.7	0.0006

Data are shown in % or mean±SD. Leptin\*: Student's t-tests were performed on log-transformed leptin. BMI=body mass index, HTN Rx=on anti-hypertensive medication, Lipid Rx=on lipid lowering drug, HDL-C=high density lipoprotein cholesterol, LDL-C=low density lipoprotein cholesterol, HbA1c=hemoglobin A1c, CRP=C-reactive protein

Table 2

Nutrient Intakes, Mean of Four 24-h Dietary Recalls per Person, by Gender-- INTERLIPID Study, Aito Town, Japan and Honolulu, Hawaii, USA

Variable	Men			Women		
	Japan	Hawaii	P	Japan	Hawaii	P
Number of People	123	88		111	94	
Total energy (kcal/day)	2386±528	2409±616	0.77	1897±378	1752±370	0.006
Protein (%kcal)	15.3±2.2	17.2±3.0	<0.0001	16.3±2.4	16.7±3.0	0.29
Total available carbohydrate (%kcal)	56.4±7.5	48.3±7.6	<0.0001	57.9±5.8	50.7±8.6	<0.0001
Total fat (%kcal)	21.6±4.7	31.6±6.3	<0.0001	25.1±4.5	31.9±7.7	<0.0001
SFA (%kcal)	5.6±1.5	9.1±2.3	<0.0001	6.8±1.72	9.5±2.7	<0.0001
MUFA (%kcal)	7.7±1.9	11.8±2.8	<0.0001	8.9±2.0	11.8±3.7	<0.0001
PUFA (%kcal)	5.8±1.4	7.4±1.8	<0.0001	6.5±1.2	7.4±2.2	0.0005
n-3 PUFA (%kcal)	1.2±0.3	0.9±0.3	<0.0001	1.3±0.4	0.9±0.3	<0.0001
n-6 PUFA (%kcal)	4.6±1.3	6.7±1.6	<0.0001	5.1±1.1	6.6±2.0	<0.0001
ARA (%kcal)	0.06±0.02	0.08±0.03	<0.0001	0.07±0.03	0.08±0.04	0.016
EPA (%kcal)	0.15±0.09	0.05±0.06	<0.0001	0.16±0.10	0.04±0.04	<0.0001
DHA (%kcal)	0.26±0.12	0.10±0.10	<0.0001	0.28±0.16	0.08±0.10	<0.0001
DPA (%kcal)	0.04±0.03	0.02±0.02	<0.0001	0.04±0.03	0.02±0.02	<0.0001
EPA+DHA+DPA (%kcal)	0.44±0.23	0.18±0.17	<0.0001	0.47±0.29	0.14±0.16	<0.0001
Dietary cholesterol (mg/1000kcal)	185.3±65.4	128.0±50.5	<0.0001	201.6±73.6	133.4±55.1	<0.0001
Total dietary fiber (g/1000kcal)	7.0±1.8	8.4±2.3	<0.0001	9.8±2.6	9.2±3.5	0.08
Keys dietary lipid score	27.2±6.1	31.2±7.8	<0.0001	30.5±6.7	32.6±9.1	0.06
Alcohol (%kcal)	6.6±6.6	2.9±4.7	<0.0001	0.7±1.7	0.6±1.62	0.88
Magnesium (mg/1000kcal)	118.4±23.1	170.0±45.4	<0.0001	142.2±26.2	160.6±43.7	0.0005
Iron (mg/1000kcal)	5.1±1.3	8.6±2.0	<0.0001	6.1±1.3	8.6±2.1	<0.0001
Phosphorus (mg/1000kcal)	523.5±89.2	587.8±122.2	<0.0001	589.3±99.7	578.1±98.4	0.42

Data are shown in mean±SD. SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids, ARA=arachidonic acid, EPA=eicosapentaenoic acid, DHA=docosahexaenoic acid, DPA=docosapentaenoic acid.

**Table 3**

Relation of Variables to Hawaii-Japan Log-transformed Serum Leptin Difference by Gender--INTERLIPID Study, Aito Town, Japan and Honolulu, Hawaii in USA.

Model	Coefficient for Site (Japan=0)	P value for Site	Coefficient for Other Variable	% change in Site Coeff. from Model 1
<b>Men</b>				
Model 1 (Site, Age)	0.245	<0.0001		
Model 1+Cigarettes/d	0.210	<0.0001	-0.0033 *(cigarettes)	14.4
Model 1 +BMI	0.0186	0.51	0.045 **(BMI)	92.4
Model 2 (Model 1+Physical)	0.212	<0.0001	-0.013 **(physical)	13.3
Model 2+ ARA	0.216	<0.0001	-0.235	11.8
Model 2+Keys dietary lipid score	0.204	<0.0001	0.00228	16.7
Model 2+ Alcohol	0.214	<0.0001	0.000348	12.7
Model 2+Fiber	0.240	<0.0001	-0.0204 **	2.0
Model 2+Iron	0.237	<0.0001	-0.00762	3.3
Model 3 (Model 2+BMI, Cigarettes/d)	0.0002	0.99		99.99
<b>Women</b>				
Model 1 (Site, Age)	0.188	<0.0001		
Model 1+Cigarettes/d	0.187	<0.0001	-0.0012(cigarettes)	0.4
Model 1 +BMI	0.100	<0.0001	0.037 **(BMI)	46.6
Model 1 +postprandial hr	0.188	<0.0001	-0.0002(postprandial hr)	0
Model 2 (Model 1+Physical)	0.188	<0.0001	-0.00006(physical)	0.1
Model 2+ ARA	0.171	<0.0001	1.3 **	9.0
Model 2+Keys dietary lipid score	0.179	<0.0001	0.0045 *	4.8
Model 2+ Alcohol	0.186	<0.0001	-0.021 *	1.1
Model 2+Fiber	0.176	<0.0001	-0.012 *	6.4
Model 2+Iron	0.188	<0.0001	-0.00032	0
Model 3 (Model 2 +BMI, Cigarettes/d)	0.0958	0.0006		49.0
Model 3+Protein	0.0952	0.0006	0.003	49.3
Model 3+Carbohydrates	0.103	0.0005	0.001	45.3
Model 3+Total fat	0.102	0.0008	-0.001	45.9
Model 3+Total n-3 PUFA	0.103	0.0011	0.017	45.2
Model 3+EP_DH_DP	0.101	0.002	0.015	46.4
Model 3+ARA	0.0938	0.0008	0.259	50.1
Model 3+Fiber	0.0931	0.0009	-0.004	50.5
Model 3+Magnesium	0.0951	0.0007	0.00003	48.4
Model 3+Iron	0.0863	0.0082	0.004	54.1
Model 3+Phosphorous	0.0972	0.0006	0.00001	48.5
Model 3+Alcohol	0.0957	0.0005	-0.016 *	49.1
Model 3+Alcohol, Fiber	0.0927	0.0008	-0.016 *(alcohol), -0.004(fiber)	50.7

Model	Coefficient for Site (Japan=0)	P value for Site	Coefficient for Other Variable	% change in Site Coeff. from Model 1
Model 3+Alcohol, Fiber, Phosphorus	0.0936	0.0007	-0.016 *(alcohol), -0.004(fiber), 0.0001(phos)	50.2
Model 3+Alcohol, Fiber, Magnesium	0.0750	0.016	-0.017 *(alcohol), -0.010(fiber), 0.0007(magn)	60.1
Model 3+Alcohol, Fiber, Iron	0.0551	0.126	-0.017 *(alcohol), -0.009(fiber), 0.014(iron)	70.7

Coefficients for multiple linear regression models used to examine the relations of lifestyle factors to Hawaii-Japan differences in log-transformed leptin concentration in men (123 in Japan and 88 in Hawaii) and women (111 in Japan and 94 in Hawaii), with control for non-dietary variables as shown.. P values for site coefficient are presented. P values for the other coefficients are indicated by

\* P<0.05,

\*\* P<0.01.

BMI=body mass index, Physical Activity=moderate or heavy physical activity, usual number of hours per day, SFA=saturated fatty acids, MUFA=monounsaturated fatty acids, PUFA=polyunsaturated fatty acids, EPA= eicosapentaenoic acid, DHA= docosahexaenoic acid, DPA=docosapentaenoic acid, ARA=arachidonic acid, EP\_DH\_DP=EPA+DHA+DPA.