DiVA – Digitala Vetenskapliga Arkivet <http://umu.diva-portal.org>

This is an author produced version of a paper published in International journal of obesity

This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

Citation for the published paper:

Mikael Lilja, Olov Rolandsson, JE Shaw, Vassen Pauvaday, Adrian Cameron, J Tuomilehto, KGMM A

Higher leptin levels in Asian Indians than Creoles and Europids: a potential explanation for increased metabolic risk

International journal of obesity, 2010, Vol. 34, Issue: 5, pp. 878-885

URL: <http://dx.doi.org/10.1038/ijo.2010.19>

Access to the published version may require subscription. Published with permission from:

[Publisher]

Higher leptin levels in Asian Indians than Creoles and Europids – a potential explanation for increased metabolic risk.

Mikael Lilja MD^{1,2}, Olov Rolandsson MD, PhD², Jonathan E Shaw MD, PhD³, Vassen Pauvaday MD⁴, Adrian J Cameron MPH, PhD³, Jaakko Tuomilehto MD, PhD⁵, KGMM Alberti MD, PhD⁶, Paul Z Zimmet MD, PhD³, Stefan Söderberg MD, PhD^{2,3}.

¹The Research and Development Unit, Jämtland County Council, Östersund, Sweden,

²Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden,

³Baker IDI Heart and Diabetes Institute, Melbourne, Australia,

⁴Ministry of Health and Quality of Life, Port Louis, Mauritius,

⁵Department of Public Health, University of Helsinki, Helsinki, Finland and South Ostrobothnia Central Hospital, Seinäjoki, Finland

⁶Department of Endocrinology and Metabolic Medicine, Imperial College, London, United Kingdom.

Running title: Higher leptin in Asian Indians.

Word count: 2929

Correspondence: Dr Mikael Lilja, Odensala Hälsocentral, Klöverv.2, SE-831 62 Östersund, Sweden. E-mail: mikael.lilja@jll.se

Phone: +4663142214, fax: +4663142221

Abstract

Background and purpose

Leptin predicts cardiovascular diseases and type 2 diabetes, diseases to which Asian Indians are highly susceptible. As a risk marker, leptin's intra-individual and seasonal stability is unstudied and only small studies have compared leptin levels in Asian Indians with other populations. The aim of this study was to explore ethnicity related differences in leptin levels and its intra-individual and seasonal stability.

Methods

Leptin and anthropometric data from the Northern Sweden MONICA (3513 Europids) and the Mauritius Non-communicable Disease (2480 Asian Indians and Creoles) studies were used. In both studies men and women 25-74 years old participated in both an initial population survey and a follow-up after 5-13 years. For the analysis of seasonal leptin variation, a subset of 1780 participants, 30-60 years old, in the Västerbotten Intervention Project was used.

Results

Asian Indian men and women had higher levels of leptin, leptin per BMI unit (leptin/BMI) or per cm in waist circumference (leptin/waist) than Creoles and Europids when adjusted for BMI (all $p < 0.0005$) or waist circumference (WC) (all $p < 0.005$). In men, Creoles had higher leptin, leptin/BMI and leptin/waist than Europids when adjusted for BMI or WC (all $p < 0.0005$). In women, Creoles had higher leptin/BMI and leptin/waist than Europids only when adjusted for WC ($p < 0.0005$). Asian Indian ethnicity in both sexes, and Creole ethnicity in men, was independently associated with high leptin levels. The intra-class correlation for

leptin was similar (0.6-0.7), independently of sex, ethnicity or follow-up time. No seasonal variation in leptin levels was seen.

Conclusion

Asian Indians have higher levels of leptin, leptin/BMI and leptin/waist than Creoles and Europids. Leptin has a high intra-individual stability and seasonal leptin variation does not appear to explain the ethnic differences observed here.

Key words: leptin, ethnicity, season, intra-individual stability.

Introduction

The impact of obesity on morbidity and mortality is well established, even though the mediators are incompletely understood.¹ Our understanding of the metabolic and endocrine importance of the adipose tissue has rapidly increased during the last decade. The adipocyte-derived hormone leptin is important for communication between adipose tissue and several metabolic pathways,^{2,3} but may also have detrimental effects on the cardiovascular system^{4,5} and the insulin-producing β -cell.⁶ We, and others have shown that leptin is an independent predictor of type 2 diabetes^{7,8} and cardiovascular disease^{4,9} and that it predicts worsening of the metabolic syndrome independently of obesity.¹⁰

The prevalence of diabetes is high among South Asians,¹¹ and the increase in numbers of people with diabetes by the year 2025 is predicted to be greatest in the countries of South-East Asia.¹² Migrants from South Asia have a higher prevalence of diabetes¹³ and coronary heart disease¹⁴ than European populations which traditional risk factors can not fully explain.¹⁴ Compared to the indigenous African population, Asian migrants in Tanzania and South Africa have a higher prevalence of diabetes.¹⁵ In Mauritius, the Asian Indian and Creole populations had a high prevalence of diabetes of 18.6 and 17.0 % respectively in 1998.¹⁶

Studies including Asian populations in which leptin levels among different ethnic groups have been compared are few in number and small in size. In a group of 140 insulin-resistant men, Asian Indians had higher leptin than Caucasians,¹⁷ and 10 lean and healthy age and BMI-matched Asian Indian men in Singapore, had higher levels of leptin than both Chinese and Caucasians.¹⁸ Comparative studies of non-Asian populations are more frequent, but mostly small in size. African Americans had higher leptin levels than non-Hispanic whites, but not Mexican Americans when controlling for anthropometric measures.¹⁹

After adjusting for fat mass, older African Americans had higher leptin levels than older white Americans, a difference attributed to a higher proportion of subcutaneous fat in African Americans.²⁰ In South Africa, African women had higher levels of leptin than Caucasian women, especially when adjusted for waist circumference (WC).²¹ A Chilean study demonstrated lower leptin levels in Mapuche natives than Caucasians²² and we have shown that Peruvian Indian women had a lower leptin to BMI ratio than Swedish women.²³ Other studies have revealed no ethnic differences.^{24,25,26} Leptin levels and signalling may thus, independently of obesity, differ between different ethnic groups, and hence potentially influence the risk for diabetes and cardiovascular disease.

A reliable risk marker should be stable over time and few data exist on the intra-individual and seasonal stability of leptin. To our knowledge, only one study on 25 young women has been published on leptin's long-term test retest stability in humans²⁷ and there are no data on potential ethnic differences in intra-individual stability.

In many seasonally breeding animals a significant seasonal fluctuation of leptin levels is described, driven by changes in photo-periodic length.²⁸ Whether such variation exists in humans is unclear. Two small longitudinal studies with eighteen,²⁹ and twenty-five³⁰ men and women respectively showed no seasonal variation in leptin levels, while a cross-sectional study on 65 male older African Americans showed slight seasonal leptin variation.³¹ The aim of this study was to utilise large population-based samples of different ethnicities to explore differences in leptin levels, and the intra-individual and seasonal stability of leptin.

Methods

Subjects

Anthropometric and leptin data from three different population surveys were used. The Northern Sweden MONICA survey took place in the two northernmost counties,

Norrbottnen and Västerbotten, and details about the survey procedures have previously been published.³² Participants from the surveys in 1986, 1990 and in 1994 were re-investigated in 1999 with the same methodology. In these surveys 250 men and 250 women randomly selected from each age group (25-34, 35-44, 45-54, and 55-64 years old) were invited and in 1994 the age group 65-74 was included. The participation rate was 79 % and 3772 out of 5129 participants were re-investigated in 1999. The population in northern Sweden is largely European with over 98 % born in Sweden or Finland. Ethnicity was not registered in the surveys, and a small fraction of the study population may therefore have been of non-European ethnicity. Leptin was analysed in 1722 men and 1823 women who participated in both baseline and follow-up surveys.

Mauritius is a sub-tropical island located in the South-western Indian Ocean with a population of about 1.2 million. The multi-ethnic population consists of 68 % Asian Indian origin, 3 % Chinese origin, and 27 % are of the general population, which mainly comprises people with mixed African and Malagasy ancestry with some European and Indian admixture (Creoles).³³ In 1987, ten randomly selected (with probability proportional to size) population clusters and one purposely-selected area of Chinatown in the capital, Port Louis, were surveyed in the Mauritius Non-communicable Disease Study. All eligible adults were invited and 5083 participated (response rate 86%). A follow-up survey was performed in 1992, which included 1111 men and 1369 women. Details on survey methodology and procedures have previously been published.^{8,16}

The MONICA and Mauritius surveys were performed during the first months of the year (MONICA in January to April and Mauritius in April to May). In contrast, the Västerbotten Intervention Program (VIP) ran throughout the year. VIP is a community intervention program targeting cardiovascular disease and diabetes prevention in the Västerbotten County in northern Sweden and subjects were invited to a health survey at their

nearest health care centre in the year they turned 30, 40, 50 and 60 years old. A cross-sectional subset of 870 men and 910 women from the Lycksele area, participating 1988 to 1992 were used in this study for the exploration of potential seasonal variation in leptin levels. The overall participation rate was 74 %.³⁴ Lycksele, situated 200 kilometres south of the polar circle, has great variation in day length throughout the year, from 4 hours in December to 21 hours in June.

Leptin was measured both in Melbourne, Australia (Mauritius samples) and in Umeå, Sweden (MONICA and VIP samples). The same test method (RIA, Linco, St. Charles, MO, USA) was used using stored samples. The lower limit of detection with this assay was 0.5 ng/mL and inter-assay and intra-assay CVs were 4.1-8.2% and 4.2%, respectively.

We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during the research. The MONICA and VIP survey protocols were approved by the Research Ethics Committee at Umeå University, Umeå, Sweden. The Mauritius survey protocols were reviewed and approved by the Alfred Healthcare Group Ethics Committee (Melbourne, Australia) as well as by the Ministry of Health, Mauritius. Informed consent was obtained from all participants of all surveys.

Statistical analysis

Due to the skewed distribution of leptin, logarithmically transformed leptin (lnleptin) was used in all analyses in addition to non-transformed leptin levels. As this did not change the outcome the transformed results are not presented.

Baseline anthropometry and leptin levels, and the changes during follow-up were explored using ANOVA. Differences in leptin levels and leptin/BMI or leptin/waist at

baseline between different ethnic groups were explored using ANCOVA adjusting for age, smoking habits and BMI or WC.

A stepwise and sex-stratified linear multivariable regression was performed to evaluate the independence of ethnicity in relation to other known determinants for leptin levels. Dummy variables were constructed and European ethnicity was the reference.

Seasonal changes in leptin levels were explored separately for men and women in different months, seasons, BMI levels and age groups using ANOVA and ANCOVA adjusting for age and BMI. Bonferroni post hoc tests were used to account for multiple comparisons.

Stability of leptin over time (“test retest stability”) was explored with intra class correlation (ICC), two-way mixed, type consistency. The results of the single measurements are presented. Furthermore, the analysis was also done taking change in BMI between initial survey and follow-up into account.

The Chinese group in Mauritius was excluded from analysis as it was judged to be too small for a meaningful analysis (35 men and 28 women). Pregnant women were excluded. All analysis was conducted using SPSS (Chicago, IL, USA) version 16.

Results

The characteristics of the Mauritius and the Swedish MONICA populations are presented in Table 1. The Swedish participants were older than the Mauritian participants and smoking habits differed substantially. Over 50 % of Asian Indian and Creole men smoked, whilst only 1 % of Asian Indian women and 16 % of Creole women were smokers. In Sweden 20 % of both Swedish men and women were regular smokers. Leptin levels adjusted for age and smoking habits were significantly higher in Asian Indians ($p < 0.0005$ for both men and women) and in Creoles ($p < 0.05$ for men and $p < 0.005$ for women) compared to Europeans.

In Table 2, levels of leptin, leptin/BMI and leptin/waist at the baseline surveys in 1987 in Mauritius and in 1986, 1990 and in 1994 in northern Sweden are presented adjusted for age and smoking habits together with either BMI or WC. After adjustment for BMI, Asian Indian men and women had significantly higher levels of leptin, leptin/BMI and leptin/waist than Creole and Europid men and women (all $p < 0.0005$). Furthermore, Creole men had higher leptin, leptin/BMI and leptin/waist than Europid men (all $p < 0.0005$). After adjustment for WC, Asian Indian men had higher leptin, leptin/BMI and leptin/waist than Europid men (all $p < 0.0005$) and Creole men ($p < 0.005$, $p < 0.0005$, $p < 0.0005$ respectively) and Asian Indian women had higher leptin, leptin/BMI and leptin/waist than both Creole and Europid women (all $p < 0.0005$). There was no difference between Creole and Europid women when adjustment was made for BMI. With adjustment for WC instead of BMI, Creole women had significantly higher leptin, leptin/BMI and leptin/waist (all $p < 0.0005$) than Europid women.

Figure 1 shows adjusted leptin/BMI levels according to ethnicity. Panel A shows leptin/BMI levels adjusted for age, smoking and BMI and panel B shows leptin/BMI levels adjusted for age, smoking and WC. Analysis with adjustment for BMI together with WC were identical and are not shown. Adding Swedish “snus” (moist snuff) to tobacco users did not change the results.

A model including age, BMI, WC, and smoking status explained 41.3 % and 50.1 % of the variance of lnleptin in men and women, respectively. Adding ethnicity could explain an extra 6.6 % ($p < 0.0005$) in men, and an extra 1.4 % ($p < 0.0005$) in women. With a stepwise method, an Asian Indian ($\beta = 0.392$; $p < 0.001$) and a Creole ($\beta = 0.183$; $p < 0.001$) background together with BMI ($\beta = 0.122$; $p < 0.001$) and age ($\beta = 0.006$; $p < 0.001$) remained associated with lnleptin in men; and in women, an Asian Indian background ($\beta = 0.165$; $p < 0.001$) and BMI ($\beta = 0.105$; $p < 0.001$) were independently associated with lnleptin levels.

Swedish males use smokeless tobacco, “snus”, a moist form of snuff, more often than cigarettes. As cigarette smoking can influence leptin levels,^{5,35} the effects of snus on leptin levels were explored specifically within the MONICA population. Males smoking at both initial survey and re-investigation, but not using snus (n=128), using snus at both occasions but not smoking (n=228) and males remaining free from tobacco at both surveys (n=1010) were chosen. Adjusted (age, observation time, baseline and change in BMI) increases of leptin (ng/mL) between baseline survey and re-investigation were 2.6 (2.0–3.3), 3.1 (2.6–3.6), and 3.0 (2.8–3.3), respectively, which did not differ between groups.

Table 3 presents the intra-individual “test retest” stability of leptin and Inleptin, measured as ICC. ICC values showed that baseline leptin values were highly correlated with follow-up values. For both men and women, all ethnic groups and with varying observation time (5 to 13 years), ICC values were comparable (range 0.59-0.70), with higher values for Inleptin (range 0.65-0.82) (all $p < 0.0001$). After adjustment for changes in BMI, the ICC for leptin in all cases was 0.75 ($p < 0.0001$).

Assessment of seasonal differences in leptin and leptin/BMI for men and women are presented in Table 4. Data were also explored for different age and BMI groups and for different seasons (not shown). No significant variation over the year was seen.

Discussion

We present the largest comparison of leptin levels in adults from different ethnic groups. Most strikingly, Asian Indian men and women have significantly higher levels of leptin compared to Creole men and women as well as to European men and women. These differences remained after adjustments for traditional measures of body fat mass (BMI and/or WC). Asian Indian ethnicity was a substantial independent contributor to the variance in leptin levels. This is consistent with the findings of previous smaller studies that showed

higher leptin levels in Asian Indian men compared to Caucasian men.^{17,18,36} To our knowledge, no previous studies comparing leptin levels in Creole and Europid populations exist. Our findings of lower leptin levels in Europids compared to Creoles of mostly African ancestry, is in line with findings comparing Caucasians to African Americans in the USA^{19,20} and to Africans in South Africa.²¹

The relationship between body fat mass and plasma leptin levels is well established,³⁷ and in both sexes the estimated subcutaneous fat mass (SCF) is a stronger predictor of leptin levels than the intra abdominal fat depot (IAF).^{38,39} Many studies have shown an increased fat mass and SCF in Asian Indian men and women at comparable BMI or waist-to-hip ratio (WHR) compared to both Europids and African Americans.^{40,41,42} The percentage of visceral to total fat seems to be similar in different populations with 17-19 % IAF in studies comparing Asian Indian men to Swedish men,⁴⁰ and to African American diabetic men.⁴³

Asian Indian non-obese men have larger adipocyte size than Europid men,³⁶ a finding which is more closely associated to hyperleptinemia than adipose tissue hyperplasia.⁴⁴ Adipocyte hypertrophy is also associated with insulin resistance,⁴⁵ and an increase of nonesterified fatty acids (NEFAs),¹⁷ and predicts type 2 diabetes.⁴⁶ It is also inversely linked to both glucose disposal rate and adiponectin levels,³⁶ which together could indicate a dysfunctional adipocyte metabolism in Asian Indians.¹⁷ It is plausible that genetic factors could influence adipocyte size, and hence leptin levels in Asian Indians as has been demonstrated in Pima Indians, where roughly half of the variance in mean subcutaneous adipocyte size was attributed to heritability.⁴⁷ To our knowledge no studies addressing adipocyte size in Creoles exist.

The high levels of leptin and leptin relative to BMI or WC, that we have shown in Asian Indians is of interest due to the linkage in both experimental⁶ and

epidemiological^{4,7,8,9} research between leptin and different features of the metabolic syndrome, as well as to disturbed adipose tissue metabolism.^{17,36}

We found a high intra-individual, test retest stability of leptin with an ICC between 0.6 to 0.7 for all examined groups. A similar ICC of 0.6 has been described in 25 young healthy women.²⁷ Test retest stability is important both when regarding leptin as a risk marker for different features of the metabolic syndrome, and to support the concept of leptin as a “defence hormone” against loss of body fat, counteracting successful weight loss and thus re-establishing previous leptin levels.⁴⁸ As a comparison, ICCs for cholesterol and LDL in men and women were 0.6 and 0.8 respectively and for triglycerides 0.6 and 0.5 respectively.⁴⁹

A marked seasonal variation in leptin levels has been demonstrated in many animals, with the variation driven by changes in photoperiodic length.²⁸ The presence of similar variation in humans should be most evident in a circumpolar setting, such as in northern Sweden. Our findings suggest that such variation is not present in humans, and that seasonal variation does not explain the ethnic differences in leptin levels observed in this study. Our finding is in line with two small longitudinal surveys,^{29,30} but opposes one smaller cross-sectional survey³¹ which showed a small correlation ($r=0.3$) by season. One weakness with our study, even though the sample size by far exceeds other studies addressing the same issue, is that we only have samples from throughout the year from separate individuals. To confirm our findings longitudinal studies of both men and women at different ages are needed.

We believe that the results presented here are likely to be generalizable to other ethnically similar populations, since these studies utilised large population-based cohorts. The lack of definite knowledge of ethnicity in the Northern Sweden MONICA is a potential weakness, however it is known that over 98 % of the survey populations were born in Sweden

or Finland. Furthermore, any misclassification of ethnicity is likely to reduce statistical power to detect differences between groups.

Leptin was analysed in two different laboratories (Melbourne and Umeå), however since the same assay was used on stored samples at both sites we do not believe this should have influenced the results observed.

We conclude that in these large population-based samples, Asian Indians have higher levels of leptin than both Creole and European populations, independent of traditional measures of obesity. We also conclude that leptin levels appear to be stable over time within individuals, with no evidence of seasonal fluctuation in the population observed, attributes that are important for a metabolic and cardiovascular disease risk marker.

Acknowledgements

We are indebted to the Northern Swedish MONICA Project, the Västerbotten Intervention Program, and the Mauritius survey, and the grants supporting them. This study was also supported by grants from the Swedish Heart and Lung foundation, the county councils of Northern Sweden (Visare Norr), the Faculty of Medicine, Umeå University. Dr Lilja was supported by the research and development unit of Jämtland county council, Östersund, Sweden and Dr. Söderberg was supported by grants from the Swedish Heart and Lung Foundation and the Swedish Society of Medicine. The authors are grateful to Ray Spark, Karin Hjertqvist and Margareta Danielsson for assistance in technical matters.

Conflict of interest

The authors declare no conflict of interest.

References

-
- ¹ Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser* 2000; **894**: i-xii,1-253.
- ² Margetic S, Gazzola C, Pegg GG, Hill RA. Leptin: a review of its peripheral actions and interactions. *Int J Obes Relat Metab Disord* 2002; **26**: 1407-1433.
- ³ Hynes GR, Jones PJ. Leptin and its role in lipid metabolism. *Curr Opin Lipidol* 2001; **12**: 321-327.
- ⁴ Beltowski J. Leptin and atherosclerosis. *Atherosclerosis* 2006; **189**: 47-60.
- ⁵ Koh KK, Park SM, Quon MJ. Leptin and cardiovascular disease: response to therapeutic interventions. *Circulation* 2008; **117**: 3238-3249.
- ⁶ Kieffer TJ, Habener JF. The adipoinsular axis: effects of leptin on pancreatic beta-cells. *Am J Physiol Endocrinol Metab* 2000; **278**: E1-E14.
- ⁷ McNeely MJ, Boyko EJ, Weigle DS, Shofer JB, Chessler SD, Leonnetti DL et al. Association between baseline plasma leptin levels and subsequent development of diabetes in Japanese Americans. *Diabetes Care* 1999; **22**: 65-70.
- ⁸ Söderberg S, Zimmet P, Tuomilehto J, Chitson P, Gareeboo H, Alberti KGMM et al. Leptin predicts the development of diabetes in Mauritian men, but not women: a population-based study. *Int J Obes* 2007; **31**: 1126-1133.
- ⁹ Söderberg S, Stegmayr B, Stenlund H, Sjöström LG, Ågren A, Johansson L et al. Leptin, but not adiponectin, predicts stroke in males. *J Intern Med* 2004; **256**: 128-136.
- ¹⁰ Franks PW, Brage S, Luan J, Ekelund U, Rahman M, Farooqi S et al. Leptin predicts a worsening of the features of the metabolic syndrome independently of obesity. *Obes Res* 2005; **13**: 1476-1484.
- ¹¹ Qiao Q, Hu G, Tuomilehto J, Nakagami T, Balkau B, Borch-Johnsen K et al. Age- and sex-specific prevalence of diabetes and impaired glucose regulation in 11 Asian cohorts. *Diabetes Care* 2003; **26**: 1770-1780.

-
- ¹² International Diabetes Federation. *Diabetes atlas, 3rd edition* 2006.
- ¹³ Mather HM, Keen H. The Southall Diabetes Survey: prevalence of known diabetes in Asians and Europeans. *Br Med J* 1985; **291**: 1081-1084.
- ¹⁴ McKeigue PM, Miller GJ, Marmot MG. Coronary heart disease in south Asians overseas: a review. *J Clin Epidemiol* 1989; **42**: 597-609.
- ¹⁵ Motal AA, Omar MA, Pirie FJ. Epidemiology of type 1 and type 2 diabetes in Africa. *J Cardiovasc Risk* 2003; **10**: 77-83.
- ¹⁶ Söderberg S, Zimmet P, Tuomilehto J, de Courten M, Dowse GK, Chitson P et al. Increasing prevalence of type 2 diabetes in all ethnic groups in Mauritius. *Diabet Med* 2005; **22**: 61-68.
- ¹⁷ Abate N, Chandalia M, Snell PG, Grundy SM. Adipose tissue metabolites and insuline resistance in nondiabetic Asian Indian men. *J Clin Endocrinol Metab* 2004; **89**: 2750-2755.
- ¹⁸ Liew CF, Seah ES, Yeo KP, Lee KO, Wise SD. Lean, nondiabetic Asian Indians have decreased insulin sensitivity and insulin clearance, and raised leptin compared to Caucasians and Chinese subjects. *Int J Obes Relat Metab Disord* 2003; **27**: 784-789.
- ¹⁹ Ruhl CE, Everhart JE. Leptin concentrations in the United States: relations with demographic and anthropometric measures. *Am J Clin Nutr* 2001; **3**: 295-301.
- ²⁰ Ruhl CE, Everhart JE, Ding J, Goodpaster BH, Kanaya AM, Simonsick EM et al. Serum leptin concentrations and body adipose measures in older black and white adults. *Am J Clin Nutr* 2004; **80**: 576-583.
- ²¹ Schutte AE, van Vuuren D, van Rooyen JM, Huisman HW, Schutte R, Malan L et al. Inflammation, obesity and cardiovascular function in African and Caucasian women from South Africa: the PIWIRS study. *J Hum Hypertens* 2006; **20**: 850-859.

-
- ²² Pérez-Bravo F, Albala C, Santos JL, Yañez M, Carrasco E. Leptin levels distribution and ethnic background in two populations from Chile: Caucasian and Mapuche groups. *Int J Obes Relat Metab Disord* 1998; **22**: 943-948.
- ²³ Lingärde F, Söderberg S, Olsson T, Ercilla MB, Correa LR, Ahrén B. Overweight is associated with lower serum leptin in Peruvian Indian than in Caucasian women: a dissociation contributing to low blood pressure? *Metabolism* 2001; **50**: 325-329.
- ²⁴ Wei M, Stern MP, Haffner SM. Serum leptin levels in Mexican Americans and non-Hispanic whites: association with body mass index and cigarette smoking. *Ann Epidemiol* 1997; **7**: 81-86.
- ²⁵ Hergenç G, Schulte H, Assman G, von Eckardstein A. Associations of obesity markers, insulin, and sex hormones with HDL-cholesterol levels in Turkish and German individuals. *Atherosclerosis* 1999; **145**: 147-156.
- ²⁶ Tanaka M, Umezaki M, Natsuhara K, Yamauchi T, Inaoka T, Hongo T et al. No difference in serum leptin concentrations between urban-dwelling Austronesians and Non-Austronesians in Papua New Guinea. *Am J Hum Biol* 2005; **17**: 696-703.
- ²⁷ Kaplan RC, Ho GY, Xue X, Rajpathak S, Cushman M, Rohan TE et al. Within-individual stability of obesity-related biomarkers among women. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 1291-1293.
- ²⁸ Rousseau K, Atcha Z, Loudon ASI. Leptin and seasonal mammals. *J Neuroendocrinol* 2003; **15**: 409-414.
- ²⁹ Donahoo WT, Jensen DR, Shepard TY, Eckel RH. Seasonal variation in lipoprotein lipase and plasma lipids in physically active, normal weight humans. *J Clin Endocrinol Metab* 2000; **85**: 3065-3068.
- ³⁰ Plasqui G, Kester AD, Westerterp KR. Seasonal variation in sleeping metabolic rate, thyroid activity, and leptin. *Am J Physiol Endocrinol Metab* 2003; **285**: E338-E343.

-
- ³¹ Perry HM 3rd, Miller DK, Patrick P, Morley JE. Testosterone and leptin in older African-American men: relationship to age, strength, function and season. *Metabolism* 2000; **49**: 1085-1091.
- ³² Stegmayr B, Lundberg V, Asplund K. The events registration and survey procedures in the Northern Sweden MONICA Project. *Scand J Public Health Suppl* 2003; **61**: 9-17.
- ³³ Central Intelligence Agency (CIA). Washington DC, USA. *The world factbook 2009* <https://www.cia.gov/library/publications/the-world-factbook/geos/mp.html> Last accessed: 8th October 2009.
- ³⁴ Rolandsson O, Hägg E, Hampe C, Sullivan EP Jr, Nilsson M, Jansson G et al. Glutamate decarboxylase (GAD65) and tyrosine phosphatase-like protein (IA2) autoantibodies index in a regional population is related to glucose intolerance and body mass index. *Diabetologia* 1999; **42**: 555-559.
- ³⁵ Reseland JE, Mundal HH, Hollung K, Haugen F, Zahid N, Anderssen SA et al. Cigarette smoking may reduce plasma leptin concentration via catecholamines. *Prostaglandins Leukot Essent Fatty Acids* 2005; **73**: 43-49.
- ³⁶ Chandalia M, Lin P, Seenivasan T, Livingston EH, Snell PG, Grundy SM et al. Insulin resistance and body fat distribution in South Asian men compared to Caucasian men. *PLoS One* 2007; **2**: e812.
- ³⁷ Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1995; **1**: 1155-1161.
- ³⁸ Dua A, Hennes MI, Hoffmann RG, Maas DL, Krakower GR, Sonnenberg GE et al. Leptin: a significant indicator of total body fat but not of visceral fat and insulin insensitivity in African-American women. *Diabetes* 1996; **45**: 1635-1637.

-
- ³⁹ Cnop M, Landchild MJ, Vidal J, Havel PJ, Knowles NG, Carr DR et al. The concurrent accumulation of intra-abdominal and subcutaneous fat explains the association between insulin resistance and plasma leptin concentrations: distinct metabolic effects of two fat compartments. *Diabetes* 2002; **51**: 1005-1015.
- ⁴⁰ Chowdhury B, Lantz H, Sjöström L. Computed tomography-determined body composition in relation to cardiovascular risk factors in Indian and matched Swedish males. *Metabolism* 1996; **45**: 634-644.
- ⁴¹ WU CH, Heshka S, Wang J, Pierson RN Jr, Heymsfield SB, Laferrère B et al. Truncal fat in relation to total body fat: influences of age, sex, ethnicity and fatness. *Int J Obes* 2007; **31**: 1384-1391.
- ⁴² Banerji MA, Faridi N, Atluri R, Chaiken RL, Lebovitz HE. Body composition, visceral fat, leptin and insulin resistance in Asian Indian men. *J Clin Endocrinol Metab* 1999;84:137-144.
- ⁴³ Banerji MA, Lebowitz J, Chaiken RL, Gordon D, Kral JG, Lebovitz HE. Relationship of visceral adipose tissue and glucose disposal is independent of sex in black NIDDM subjects. *Am J Physiol* 1997; **273**: E425-E432.
- ⁴⁴ Couillard C, Mauriège P, Imbeault P, Prud'homme D, Nadeau A, Tremblay A et al. Hyperleptinemia is more closely associated with adipose cell hypertrophy than with adipose tissue hyperplasia. *Int J Obes Relat Metab Disord* 2000; **24**: 782-788.
- ⁴⁵ Lundgren M, Svensson M, Lindmark S, Renström F, Ruge T, Eriksson JW. Fat cell enlargement is an independent marker of insulin resistance and "hyperleptinemia". *Diabetologia* 2007;**50**:625-633.
- ⁴⁶ Weyer C, Foley JE, Bogardus C, Tataranni PA, Pratley RE. Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts type II diabetes independent of insulin resistance. *Diabetologia* 2000;**43**:1498-1506.

⁴⁷ Weyer C, Wolford JK, Hanson RL, Foley JE, Tataranni PA, Bogardus C et al.

Subcutaneous abdominal adipocyte size, a predictor of type 2 diabetes, is linked to chromosome 1q21-q23 and is associated with common polymorphism in LMNA in Pima Indians. *Mol Genet Metab* 2001;**72**:231-238.

⁴⁸ Flier JS. Clinical review 94: What's in a name? In search of leptin's physiologic role. *J Clin Endocrinol Metab* 1998; **83**: 1407-1413.

⁴⁹ Al-Delaimy WK, Jansen EH, Peeters PH, van der Laan JD, van Noord PA, Boshuizen HC et al. Replicability of biomarkers of iron status, blood lipids, oxidative stress, vitamin D, C-reactive protein and fructosamine in two Dutch cohorts. *Biomarkers* 2006; **11**: 370-382.

Titles and legends to figures

Figure 1:

Leptin (ng/mL) per BMI (kg/m^2) unit (Leptin/BMI) for Asian Indians, Creoles and Europids adjusted for age, smoking habits together with BMI (A) or waist circumference (B). The width of the CI's for both block A and B is for men 0.02, 0.02, 0.01 and for women 0.03, 0.06 and 0.03 respectively for Asian Indians, Creoles and Europids.

Table 1. Study base

Ethnicity	Asian Indian	Creole	Europid	Europid	Europid
Survey years	1987-1992	1987-1992	1986-1999	1990-1999	1994-1999
<u>MEN</u>					
N	824	252	575	475	670
Age span, years	25-74	25-74	25-64	25-64	25-74
Age, years	39.8 (10.7)	41.6 (12.2)	44.8 (10.7)	45.5 (10.8)	51.3 (13.4)
Smokers %	53,5	59,9	19,3	19,8	14,6
Leptin, ng/ml	4.7 (3.4)	4.0 (3.1)	4.3 (3.0)	4.2 (3.1)	5.1 (3.7)
Waist, cm	78.6 (8.8)	78.0 (9.0)	92.7 (9.1)	91.0 (8.8)	93.6 (9.7)
BMI, kg/m ²	23.2 (3.4)	23.5 (3.5)	25.5 (3.3)	25.7 (3.2)	26.3 (3.6)
Hip, cm	88.1 (7.0)	88.5 (7.0)	97.8 (6.0)	97.8 (5.9)	99.7 (6.5)
Leptin/BMI	0.19 (0.13)	0.16 (0.12)	0.16 (0.10)	0.16 (0.10)	0.19 (0.11)
Leptin/waist	0.058 (0.039)	0.049 (0.037)	0.045 (0.027)	0.045 (0.029)	0.053 (0.033)
D-leptin, ng/mL	1.4 (3.1)	1.1 (2.6)	3.2 (3.9)	2.9 (3.7)	3.0 (4.7)
D-Waist, cm	8.2 (4.9)	9.2 (4.6)	4.4 (6.0)	5.5 (5.5)	3.2 (5.4)
D-BMI, kg/m ²	0.9 (1.7)	1.4 (1.7)	1.4 (2.0)	1.1 (1.7)	0.6 (1.5)
D-Hip, cm	6.0 (4.3)	8.3 (4.4)	5.5 (4.7)	5.3 (4.1)	3.7 (4.3)
<u>WOMEN</u>					
N	990	350	577	525	691
Age span, years	25-74	25-74	25-64	25-64	25-74
Age, years	41.0 (11.6)	44.4 (13.1)	44.3 (10.8)	45.3 (10.9)	50.5 (13.3)
Smokers %	1,0	16,0	26,1	22,1	22,0
Leptin, ng/mL	14.9 (12.2)	13.0 (8.1)	12.9 (8.7)	12.1 (7.5)	13.7 (8.2)
Waist, cm	74.1 (11.0)	78.0 (11.7)	84.8 (11.9)	79.1 (9.9)	84.0 (11.9)
BMI, kg/m ²	24.2 (4.6)	25.4 (5.0)	24.8 (4.3)	24.9 (4.1)	25.7 (4.5)
Hip, cm	91.8 (8.9)	94.3 (9.4)	98.2 (8.5)	97.8 (8.4)	100.5 (8.6)
Leptin/BMI	0.58 (0.40)	0.49 (0.24)	0.49 (0.27)	0.46 (0.22)	0.51 (0.24)
Leptin/waist	0.193 (0.137)	0.161 (0.085)	0.145 (0.082)	0.147 (0.076)	0.158 (0.079)
D-leptin, ng/mL	5.5 (10.8)	6.4 (9.2)	9.1 (11.5)	8.4 (10.3)	7.6 (9.6)
D-Waist, cm	8.0 (6.7)	8.2 (6.1)	2.1 (8.1)	6.9 (7.1)	1.8 (7.5)
D-BMI, kg/m ²	1.4 (2.1)	1.6 (2.2)	2.1 (2.6)	1.7 (2.3)	0.8 (1.8)
D-Hip, cm	5.6 (5.0)	5.8 (4.9)	5.8 (5.6)	6.1 (5.7)	2.8 (5.0)

Characteristics for the Mauritius survey in 1987 with reinvestigation in 1992 and the northern Sweden MONICA with surveys in 1986, 1990 or in 1994, all with reinvestigation in 1999.

Numbers, percentages, and means (SE) are presented. Pregnant women were excluded.

BMI=body mass index, Leptin/BMI=leptin (ng/mL) per BMI (kg/m²) unit, leptin/waist=leptin per cm in waist circumference. D-leptin, D-waist, D-BMI, D-hip= change between first survey and re-investigation in leptin, waist circumference, BMI and hip circumference respectively.

Table 2. Leptin levels and ethnicity

		Asian Indian	Creole	Europid	p, post hoc
<u>MEN</u>					
N		821	251	1713	
Leptin, ng/mL	†	5.79 (0.10)	4.88 (0.17)	3.94 (0.07)	***abc
Leptin, ng/mL	‡	6.72 (0.10)	6.13 (0.17)	3.31 (0.07)	***bc**a
Leptin/BMI	†	0.22 (0.004)	0.19 (0.006)	0.15 (0.003)	***abc
Leptin/BMI	‡	0.25 (0.004)	0.22 (0.006)	0.14 (0.003)	***abc
Leptin/waist	†	0.067 (0.001)	0.057 (0.002)	0.042 (0.001)	***abc
Leptin/waist	‡	0.075 (0.001)	0.067 (0.002)	0.037 (0.001)	***abc
<u>WOMEN</u>					
N		980	347	1787	
Leptin, ng/mL	†	15.78 (0.24)	12.33 (0.38)	12.62 (0.17)	***ab
Leptin, ng/mL	‡	17.61 (0.25)	13.82 (0.40)	11.34 (0.18)	***abc
Leptin/BMI	†	0.60 (0.01)	0.47 (0.01)	0.48 (0.01)	***ab
Leptin/BMI	‡	0.64 (0.01)	0.51 (0.01)	0.45 (0.01)	***abc
Leptin/waist	†	0.199 (0.003)	0.155 (0.004)	0.148 (0.002)	***ab
Leptin/waist	‡	0.213 (0.003)	0.167 (0.005)	0.138 (0.002)	***abc

Numbers and means (SE) are shown for leptin (ng/mL), leptin (ng/mL) per BMI unit (kg/m²) (leptin/BMI) and leptin (ng/mL) per cm in waist circumference (leptin/waist). Data are adjusted for age, smoking status and BMI (†), or age, smoking status and waist circumference (‡). Pregnant women were excluded.

** = p<0.005, *** = p<0.0005.

Post hoc: a = Asian Indians – Creoles, b = Asian Indians – Europids, c = Creoles – Europids.

Table 3. Single measures intra class correlation (ICC) for leptin.

	N	<u>leptin</u>	95% CI	<u>Inleptin</u>	95% CI
		ICC		ICC	
All cases	5930	0.70	0.68-0.71	0.82	0.81-0.83
All men	2796	0.61	0.58-0.63	0.71	0.69-0.73
All women	3134	0.61	0.59-0.63	0.69	0.67-0.70
All male Asian Indians	824	0.66	0.62-0.70	0.70	0.67-0.74
All male Creoles	252	0.70	0.63-0.76	0.72	0.65-0.77
All male Europids	1720	0.59	0.56-0.62	0.73	0.70-0.75
All female Asian Indians	991	0.63	0.59-0.67	0.67	0.64-0.70
All female Creoles	350	0.65	0.58-0.70	0.75	0.70-0.79
All female Europids	1793	0.60	0.57-0.63	0.69	0.66-0.71
Male Europids 1986-1999	575	0.58	0.52-0.63	0.70	0.66-0.74
Male Europids 1990-1999	475	0.62	0.60-0.67	0.74	0.69-0.78
Male Europids 1994-1999	670	0.58	0.53-0.63	0.75	0.71-0.78
Female Europids 1986-1999	577	0.57	0.51-0.62	0.69	0.64-0.73
Female Europids 1990-1999	525	0.56	0.50-0.62	0.65	0.60-0.70
Female Europids 1994-1999	691	0.65	0.60-0.69	0.71	0.67-0.75

Single measures intra class correlation (ICC) and 95% CI for leptin (ng/mL) and logarithmically transformed leptin (Inleptin) from first survey and reinvestigation in Mauritius (1987 and 1992 respectively) and in Northern Sweden (1986, 1990, 1994 and 1999 respectively). All women pregnant at survey or reinvestigation were excluded.

Table 4. Seasonal leptin levels.

Month	<u>MEN</u>			<u>WOMEN</u>		
	N	leptin (SE)	leptin/BMI (SE)	N	leptin (SE)	leptin/BMI (SE)
January	34	3.41 (0.48)	0.13 (0.02)	37	12.08 (1.23)	0.46 (0.04)
February	72	4.36 (0.33)	0.16 (0.01)	75	12.90 (0.87)	0.49 (0.03)
March	91	3.91 (0.29)	0.15 (0.01)	61	12.37 (0.96)	0.46 (0.03)
April	92	3.97 (0.29)	0.15 (0.01)	93	12.00 (0.78)	0.46 (0.02)
May	94	4.05 (0.29)	0.15 (0.01)	87	13.04 (0.80)	0.48 (0.02)
June	81	3.33 (0.31)	0.13 (0.01)	77	12.19 (0.85)	0.47 (0.03)
July						
August	44	3.57 (0.42)	0.14 (0.01)	45	11.94 (1.12)	0.45 (0.03)
September	84	4.09 (0.30)	0.15 (0.01)	108	11.72 (0.72)	0.45 (0.02)
October	90	3.65 (0.29)	0.14 (0.01)	116	12.27 (0.70)	0.46 (0.02)
November	100	3.76 (0.28)	0.14 (0.01)	118	13.14 (0.70)	0.49 (0.02)
December	88	4.36 (0.30)	0.16 (0.01)	93	11.46 (0.78)	0.45 (0.02)

Means (SE) for leptin (ng/mL), and leptin (ng/mL) per BMI unit (kg/m²) (leptin/BMI) adjusted for age and stratified for survey month and sex are shown. Participants were men and women aged 30, 40, 50 and 60 years old from Västerbotten County in northern Sweden.

Survey years were 1988-1992. Number of participants (men/women) aged 30 years: 285/227, 40 years: 278/271, 50 years: 194/236 and 60 years: 113/176. No samples drawn during July.