

A pilot study on hyperinsulinaemic euglycaemic clamp based insulin sensitivity in young adult Indian males with low body mass index

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Background & objectives: Indians have decreased insulin sensitivity (IS) and a greater adiposity at a lower body mass index (BMI) when compared with other ethnic groups. Despite this, IS has not been studied in Indians of low BMI. This study thus used the hyperinsulinaemic euglycaemic clamp (HEC) technique to compare IS in young normal weight (NW) and low BMI (LBMI) Indian males. Clamp IS was also compared with convenient indices of insulin sensitivity such as the homeostatic model assessment (HOMA). In the NW group, clamp IS was compared with published data of similarly measured IS in other studies and ethnic groups.

Methods: Ten NW [body mass index (BMI): 18.5-25 kg/m²] and ten LBMI (BMI < 18.5 kg/m²) young healthy Indian males aged between 19-32 yr were recruited through advertisements from Bangalore slums. Fasting plasma glucose and insulin, glucose disposal rates (GDR) and IS were the parameters measured during the HEC technique.

Results: The NW group had a Clamp IS of 4.5 (3.8, 5.3) (median, lower, upper quartile, mg/(kg. min)/μU/ml) that was close to half that of the LBMI group; 9.9 (7.1, 13.4; $P < 0.001$). Clamp IS in the NW group was significantly lower than that observed in published studies involving other ethnic groups ($P < 0.05$). Clamp IS and per cent body fat (% BF), were significantly and negatively correlated ($n = 20$, $\rho = -0.7$, $P < 0.001$). Correlations between Clamp IS and other IS indices ranged from $\rho = -0.5$ for HOMA2-%B to $\rho = 0.5$ for HOMA2-%S ($P < 0.05$); however, the correlation with HOMA1-IR was not significant ($\rho = 0.4$).

Interpretation & conclusions: The significantly lower Clamp IS of the NW group compared with the LBMI group and other ethnic groups indicated that IS was impaired in Indians at relatively low BMIs. Most of the convenient indices of IS were significantly correlated with Clamp IS, however, the Clamp IS was more sensitive method with greater discriminatory power, since IS differences between LBMI and NW groups were only apparent with Clamp IS.

Key words HOMA - hyperinsulinaemic euglycaemic clamp - insulin resistance - insulin sensitivity - percentage body fat

There are currently approximately 40.9 million patients with diabetes mellitus in India and this number is expected to rise to about 69.9 million by the

year 2025¹. There are considerable epidemiological data from migrant Indians suggesting that Indians are less insulin sensitive than other ethnic groups^{2,3}. One

reason for the decreased insulin sensitivity (IS) could be due to the accumulation of body fat, linked to a positive energy balance³⁻⁵. In India, a positive energy balance could result from a nutritional and lifestyle transition associated with high fat intakes, coupled with a low physical activity⁴⁻⁶. Indians certainly seem to have a relatively greater adiposity at a lower body mass index (BMI) when compared with other ethnic groups, both within and outside Asia⁷⁻⁹. Further, measures of overall obesity and the location of body fat are strongly associated with IS in migrant Indians in other countries^{7,10}. While there are some studies on IS using the clamp method in migrant Indians abroad^{2,3,7}, we are unaware of any studies that have been done in India. Though several epidemiological studies have used surrogates of insulin sensitivity based on fasting glucose and insulin levels in the form of homeostasis model assessment (HOMA) and quantitative insulin sensitivity check index (QUICKI)¹¹⁻¹³, these have not been formally validated in an Indian setting. Indians in India are unique in that approximately 30 per cent of adult Indians have a low BMI of < 18.5 kg/m² (Ref.14) and only 12 to approximately 15 per cent are overweight or obese¹⁵. In addition, incident diabetics in India are often of relatively low BMI¹⁶. There are no published cohort data that would quote the incidence figures. People in urban slums in India are traditionally considered loci of poverty and malnutrition indicating that the epidemiological transition in this group is already under way. It would be interesting to find out the relation between IS and anthropometric variables in this group because the body composition profile of even the low BMI group suggests that they are at increased risk of developing diabetes.

We undertook this study with three objectives. First, to describe the methodology and issues related to setting up a hyperinsulinaemic euglycaemic clamp (HEC) technique and to use this method to compare the IS of young adult male low BMI (LBMI) subjects (BMI < 18.5 kg/m²) with age and gender matched normal weight (NW) subjects (BMI between 18.5 and < 25 kg/m²). Second, to compare the rate of glucose metabolized and clamp IS of these subjects with published data of migrant Indians and other ethnic groups. Third, to compare clamp IS with other, more conveniently obtained IS indices from fasting glucose and insulin.

Material & Methods

Subjects: Ten young resident south Indian male NW and ten LBMI (aged 19-32 yr) subjects participated

in this study conducted from March 2006 to February 2007. They were recruited through advertisements in and around the St. John's Medical College campus and nearby urban slums. The purpose of the study and the potential risks involved were explained to each subject and a written informed consent was obtained from each of them. Subjects with family history of diabetes, and on medication were excluded. None of the subjects had frank diabetes using a fasting blood glucose cut-off of as 110 mg/dl, although two subjects in LBMI and one subject in NW group had impaired fasting plasma glucose using recent guidelines¹. Food intake was assessed using food diary for 3 days, and physical activity level (PAL) was calculated using 3 day physical activity diaries with activities filled in blocks of 10 min. PAL was computed as the estimated total 24 h energy expenditure from physical activity diary divided by the estimated basal metabolic rate (from standard regression equations)¹⁷. The subjects on high meat protein diet and high PAL were excluded. The subjects recruited in both the group belonged to class II socio-economic group (modified Kuppaswamy's socioeconomic status scale)¹⁸. All were in good health as determined by medical history, physical examination, blood cell counts, routine blood biochemical profile and urinalysis. Oral glucose tolerance test (OGTT) was not done in our subjects, since according to WHO¹⁹ and American Diabetes Association 2003²⁰, normal fasting plasma glucose ranged between 70 – 110 mg/dl. The Institutional Ethical Review Board of St. John's Medical College, Bangalore approved the research protocol.

Anthropometry: Anthropometric and skinfold thickness measurements were carried out on the day of the experiment early in the morning. Subjects were weighed in minimal clothing to the nearest 0.1 kg and their height was measured to the nearest 0.1 centimeter. Skinfolds (biceps, triceps, subscapular, and suprailiac) were measured to the nearest 0.2 mm using skinfold caliper (Holtain, Crymych, UK) and body density determined using age and gender specific prediction equations; body fat proportion was then calculated from body density²¹⁻²³. Since most of the muscle mass is appendicular, and earlier studies have shown that the limb girth corrected for skinfold appears to correlate best with total body skeletal mass, whole body muscle mass in the present study was predicted from an Indian equation based on skinfold corrected arm muscle area (CAMA)^{24,25}. Waist and hip circumferences were measured using a standard non stretchable tape

measure, at the narrowest point between the iliac crest and ribcage (waist) and at the level of the greater trochanter (hip).

Hyperinsulinaemic euglycaemic clamp: Subjects reported in the evening prior to the study and stayed overnight in the laboratory after being provided a standard dinner. At 0630 h after an overnight fast of 10 h, an intravenous catheter (Jelco, 22G, Medex Medical Ltd, Lancashire, UK) was inserted, under sterile precautions, into the antecubital vein for infusion of insulin and 25 per cent dextrose solutions, while another catheter was inserted in an anti-flow direction into the dorsal vein of the contra lateral hand for arterialized blood sampling (using a warm box into which the hand was placed, maintained between 60 and 65°C). A slow intravenous drip of isotonic saline was used to keep the blood sampling catheter patent.

An insulin infusate of 300 mU/ml (SI: 2083.5 μ M/l) was prepared from regular human insulin (Eli Lilly & Co, Gurgaon, India) in 100 ml isotonic saline and 4 ml of the subject's blood (previously drawn). A sterile 25 per cent dextrose solution was used to prevent hypoglycaemia during the hyperinsulinaemic euglycaemic clamp as described earlier²⁶. Insulin and glucose infusions began 30 min after cannulation, after blood samples were collected for baseline glucose and insulin measurements and the basal heart rate and blood pressure were recorded.

The protocol for performing the clamp was as described earlier²⁶. Briefly, a 10 min priming insulin infusion was followed by a constant infusion for the next 110 min at the rate of 40 mU/m² (SI: 277.80 pM/m²) surface area/min, by a calibrated infusion pump (Harvard Infusion Pump, 55-2222, Holliston, USA), to increase the plasma insulin concentration to about 100 μ U/ml (694.50 pM/l). The glucose infusion, also delivered by a Harvard Infusion pump, was begun at the 4th minute and the infusion rate was empirically set at 2.0 mg/kg/min (SI: 0.01 mM/kg/min) up to 10th minute. Subsequent glucose infusion rates were based on arterialized plasma glucose values obtained every 5 min. Plasma glucose concentration was maintained at an average basal value of 90 mg/dl (SI: 5.00 mM/l). The blood samples for glucose measurements were drawn into ethylene diamine tetra acetate (EDTA) tubes (Becton Dickinson, Singapore) and the separated plasma analyzed by the glucose oxidase method on a bedside glucose analyzer (GM9D, Analox instruments, London, UK). The intra and inter-assay coefficients of

variation for this method [using 144.1 mg/dl (8mM/l) standards] were <1 and <5 per cent respectively.

Blood samples for insulin measurements were collected every 20 min in heparinized tubes (Becton Dickinson, Singapore) and plasma stored at -80°C until analysis, by an enzyme-linked immunosorbent assay (ELISA) method (Mercoia Insulin ELISA, Uppsala, Sweden). The intra- and inter-assay coefficients of variation for this method (using tri-level lyophilized serum controls (Biorad, Irvine, CA)) were <5 and <10 per cent respectively. Glucose disposal rate and IS were calculated over 40 to 120 min of the clamp, as previously described²⁶. Mean steady state plasma glucose values, glucose infusion rates and insulin values obtained during the study are shown in Fig. 1, pannel A and B.

Other insulin sensitive indices calculated were the HOMA and QUICKI. The original HOMA model contained simple equations based on fasting plasma glucose (FPG, mM/l) and fasting plasma insulin

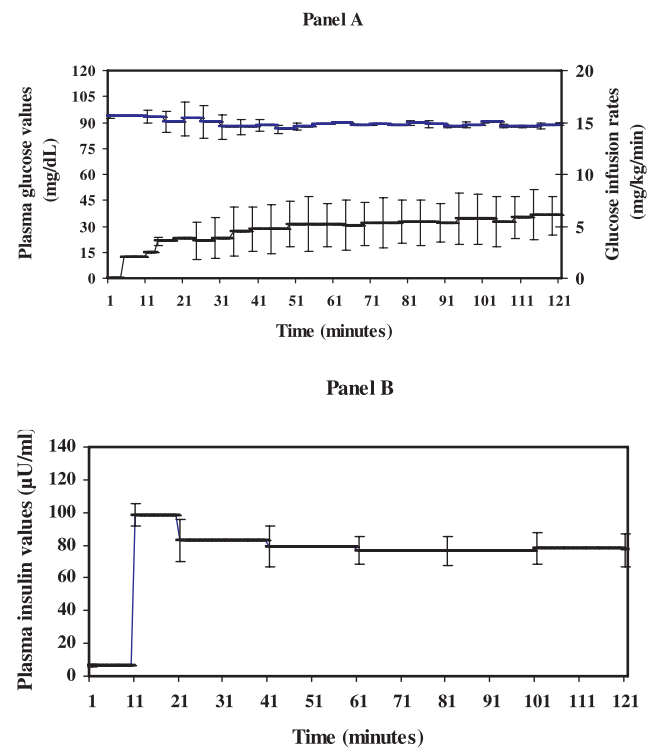


Fig. 1. Panel A: Plasma glucose and glucose infusion rates over the 120 min period of the hyperinsulinaemic euglycaemic clamp (n=20). Primary Y-axis: plasma glucose values (mg/dl), Secondary Y-axis: glucose infusion rates (mg/kg/min). Bars represent SD. **Panel B:** Plasma insulin levels (μ U/ml) during the 120 min period of the hyperinsulinaemic euglycaemic clamp (n=20). Bars represent SD.

(FPI, mU/l) concentrations, such that HOMA1-IR = (FPI x FPG)/22.5 and HOMA1-%B = (20 x FPI)/(FPG - 3.5), could be calculated to measure IR and β -cell function respectively^{11,12}. A newer software based calculation, HOMA2, can also be used to determine insulin sensitivity (HOMA2-%S) and β -cell function (HOMA2-%B) from FPG (mM/l) and total or specific insulin concentrations (pM/l). These equations are adjusted for use with newer insulin assays. The reciprocal of sensitivity expressed as HOMA2-%S, gave a value for IR in this model (HOMA2-IR)^{11,12,27}. The QUICKI is defined by the formula: $1/(\log I_0 + \log G_0) = 1/[\log(I_0 \times G_0)]$ where I_0 is fasting plasma insulin (μ U/ml) and G_0 is fasting plasma glucose (mg/dl)^{13,27,28}.

Statistical analysis: Normality of data was assessed by evaluating the ratio of skewness and the standard error of skewness. All data were expressed as median (lower, upper quartiles), since some variables did not follow a normal distribution. The studied variables were Clamp IS, glucose metabolism, BMI, %BF, waist hip ratio, HOMA1-IR, HOMA1-%B, HOMA2-%B, HOMA2-%S, HOMA2-IR and QUICKI. Since HOMA1-IR, HOMA2-%S and QUICKI were not normally distributed, non-parametric statistics were used in general. Differences in Clamp IS and other parameters between groups were assessed by the Mann-Whitney U test. The parameters to estimate IS have been compared between the groups after adjusting for height, weight and waist hip ratio (in separated models, the covariates being highly correlated with each other) using ANCOVA. Spearman's correlations were performed between Clamp IS and anthropometry or other IS indices with the entire sample (n = 20) as existing literature does not suggest a different relationship between the variables in the 2 study groups. Results were considered significant if $P < 0.05$. Regression analyses were also performed between Clamp IS and body composition (% BF), as well as the more convenient IS measurements such as the HOMA indices and QUICKI. To compare the Clamp IS in the present study with earlier published values in other ethnic groups, a two-sample t test was used after examining the homogeneity of variance. All statistical analyses were performed using SPSS (v13.0, SPSS, Chicago, USA).

Results

The age and anthropometry of the subjects are described in Table I. NW subjects had a % BF ranging from 13.6 to 25.1 per cent and LBMI from 5.4 to 12.7

per cent. The BMI of the subjects in NW and LBMI ranged from 21.8 to 24.7 and 14.7 to 17.4 kg/m² respectively. There was a significant difference in % BF, fat mass (FM), fat free mass (FFM) and waist circumference between the 2 groups ($P < 0.001$).

The fasting plasma basal glucose ranged from 82.9 to 108.8, and 87.0 to 107.5 (mg/dl) in NW and LBMI subjects respectively. The range of fasting plasma insulin in NW and LBMI subjects were from 2.8 to 9.6 and 0.7 to 10.9 (μ U/ml) respectively. The subject with low plasma insulin level was not included for HOMA and QUICKI calculations. The median (lower, upper quartile) values of steady state plasma insulin (from 40 to 120 min) during the HEC technique were 80.8 (72.0, 95.6) and 69.5 (63.6, 75.0) μ U/ml in NW and LBMI subjects respectively; these were significantly different ($P < 0.05$). The plasma glucose level was clamped at 89.2 (87.3, 91.5) and 88.0 (85.5, 90.1) mg/dl in NW and LBMI subjects. There was a significant difference in Clamp IS between the groups ($P < 0.001$). The median (lower, upper quartiles) Clamp IS value was 4.5 (3.8, 5.3) and 9.9 (7.1, 13.4) (mg/(kg.min)/ μ U/ml) for the NW and LBMI subjects, respectively, (Table II). ANCOVA for clamp IS (multiplied by weight) between groups with, height, weight and waist hip ratio as a covariate showed that there was no significant difference between the groups. However, none of the HOMA parameters or QUICKI values differed significantly between the two groups.

A simple regression analysis with Clamp IS and % BF was significant ($R^2 = 0.5$, $P < 0.001$, Fig 2). The

Table I. Anthropometric and physical activity patterns of the study subjects in the two groups

Characteristic	Low BMI (LBMI)	Normal weight (NW)
	Median (Q1,Q3)	Median (Q1,Q3)
Age (yr)	20 (19,24)	22 (20,25)
Height (m)	1.6 (1.6,1.7)	1.7 (1.7,1.8)*
Weight (kg)	44.3 (42.9,45.5)	65.0 (62.8,74.3)**
BMI (kg/m ²)	16.5 (15.8,16.9)	23.0 (22.4,23.8)**
% Body fat	8.5 (8.0,9.0)	17.9 (15.3,23.0)**
Fat mass (kg)	3.6 (3.5,3.8)	11.8 (9.9,15.8)**
% Fat free mass	91.5 (91.0,92.0)	82.1 (77.0,84.7)**
% Muscle mass	51.3 (50.2, 52.0)	47.9 (46.1,50.0)**
Waist circumference (cm)	59.2 (57.4,60.8)	76.3(72.8,80.8)**
Waist hip ratio	0.77 (0.76,0.80)	0.83 (0.81,0.85)**
PAL	1.43 (1.31,1.51)	1.44 (1.30,1.59)

n =10 in each group, BMI: body mass index, PAL: physical activity level, (Q1,Q3) = (lower quartile, upper quartile). * $P < 0.05$, ** $P < 0.001$ compared to LBMI group

Table II. Glucose disposal rates, insulin sensitivity, fasting glucose and insulin values in the two study groups

Parameter	Low BMI (LBMI)	Normal Weight (NW)
	Median (Q1,Q3)	Median (Q1,Q3)
Glucose disposal rate [mg/(kg.min)]	6.4 (5.1,8.8)	3.5 (3.2,4.5)**
Clamp IS [mg/(kg.min)]/ (μ U/ml)	9.9 (7.1,13.4)	4.5 (3.8,5.3)**
Fasting plasma insulin (μ U/ml)	5.8 (3.8,8.5)	8.0 (5.7,8.8)
Baseline Glucose (mg/dl)	90.2 (88.9,99.7)	92.2 (86.2,98.0)
HOMA1-IR	0.8 (0.5,1.2)	0.6 (0.5,0.7)
HOMA1-%B	68.3 (54.1,88.9)	111.6 (62.8,134.7)
HOMA2-%B	71.0 (60.3,84.7)	99.0 (66.2,108.8)
HOMA2-%S	133.1 (88.9,204.9)	96.2 (88.1,132.6)
HOMA2-IR	0.8 (0.5,1.1)	1.0 (0.8,1.1)
QUICKI	3.2 (3.0,3.5)	3.1 (3.0,3.3)

IS: insulin sensitivity, HOMA: homeostasis model assessment, QUICKI: quantitative insulin check index, (Q1,Q3) = (lower quartile, upper quartile); Conversion factor (CF) for SI: Glucose disposal rate = $5.55 [\mu\text{M}/(\text{kg}\cdot\text{min})]$; Clamp IS = $0.7991 (\mu\text{M}/[\text{kg}\cdot\text{min}])/(\text{pM/l})$; fasting plasma insulin = 6.945 pM/l ; Baseline glucose = 0.0555 mM/l . ** $P < 0.001$ compared to LBMI group

Clamp IS also had significant negative correlations with other anthropometric indices (BMI, % BF, fat mass (FM), waist circumference ($\rho = -0.7, -0.7, -0.8,$ and -0.7 respectively, all $P < 0.01$) and a significant positive correlation with per cent fat free mass, per cent muscle mass and physical activity level ($\rho = 0.8$ ($P < 0.001$), 0.5 ($P < 0.05$) and 0.4 , ($P < 0.05$) respectively).

Clamp IS had a significant negative correlation with HOMA1-%B, HOMA2-%B and HOMA2-IR ($\rho = -0.5, -0.6,$ and -0.5 respectively, all $P < 0.05$) and significant positive correlation with HOMA2-%S ($\rho = 0.5$, $P < 0.05$). There was no significant correlation of Clamp IS with HOMA1-IR and QUICKI ($\rho = 0.4$ and 0.3 respectively).

A survey of studies on adult humans performed using the HEC in different ethnic groups are described in Table III. Clamp IS was normally distributed in the study population and the rate of glucose disposal and Clamp IS in NW subjects were compared with other ethnic groups or with Asian Indians in other studies. Only reported parameters were compared, rather than recalculated parameters (Table III), and therefore, more comparisons were possible for glucose disposal rates

rather than IS. The glucose disposal rate observed in the NW group of this study was significantly lower than in that observed in Asian Indians in two of the studies^{2,29} ($P < 0.05$, $P < 0.001$ respectively) but higher than that in one study³ ($P < 0.05$) and not different from one study⁷ ($P = 0.2$). Similarly, with respect to comparisons of data from present study subjects with Caucasians, the mean glucose disposal rate of the Caucasians was higher in 2 of the studies^{2,29} ($P < 0.001$ for both comparisons), but comparable in one³. Interestingly, a subgroup analysis in this study³ for Caucasian subjects with per cent body fat < 20 per cent (making for more comparable body fat to the present study subjects), showed that Caucasians had a higher glucose disposal rate, while the migrant Asian Indians became more comparable to the present study NW subjects (Table III). The general trend of these findings reflected the inter-ethnic group comparisons within each literature report, which showed that Asian Indians had lower glucose disposal during an HEC than other ethnic groups. In addition, the Clamp IS was significantly lower than that reported for Asian Indians from Singapore as well as in Chinese and Caucasians²⁹ ($P < 0.05$).

Discussion

Our study demonstrated that healthy NW Indians had a reduced Clamp IS, when compared with LBMI Indians, who conceivably represent that portion of the population least affected by the nutrition and epidemiological transition that is associated with increased diabetes. This finding may be due to the differences in anthropometry and body composition. The entire sample size was considered for the correlation/regression analysis, since we were interested in the biological response along a continuum of anthropometric variables with insulin sensitivity.

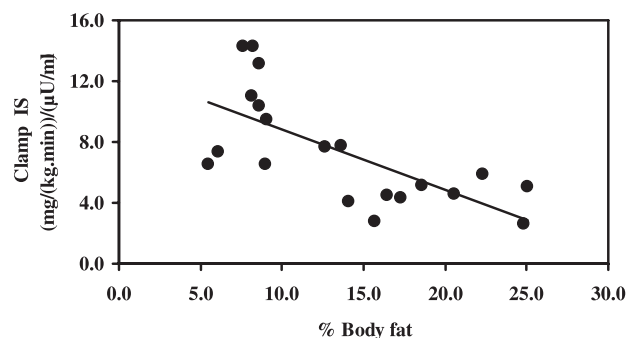


Fig. 2. Relationship between % Body fat and Clamp IS (mg/(kg.min))/(μ U/ml) for the pooled data ($n=20$). Equation: $y = -0.3953x + 12.737$, $R^2 = 0.4689$.

Table III. Glucose disposal rates and insulin sensitivity using the hyperinsulinaemic euglycaemic clamp: a comparison of the current data (mean values \pm SD) with comparative data from published studies of other ethnic groups

Study / Ethnic group	n	Age (yr)	BMI (kg/m ²)	% body fat	Basal plasma insulin (μ U/ml)	Glucose metabolism*	Clamp IS*
Present study							
Asian Indian (NBMI)	10	23	23	19	7.3 \pm 2.1 [†]	4.0 \pm 0.9 ^d 5.0 \pm 1.1 ^e 21.8 \pm 5.0 ^f	6.2 \pm 2.0 ⁱ 6.1 \pm 2.0 ^j
Raji <i>et al</i> ²							
Asian Indian	12	34	23	29 ^a	17.6 \pm 2.0 [‡]	4.7 \pm 0.4 ^d	
Caucasian	12	35	24	26 ^a	9.0 \pm 1.0 [‡]	7.5 \pm 0.3 ^d	
Chandalia <i>et al</i> ³							
Asian Indian	21	35	24	20 ^b	17.0 \pm 4.0 [‡]	3.7 \pm 1.3 ^e	6.0 \pm 1 ^{ik}
Caucasian	23	40	25	19 ^b	14.0 \pm 4.0 [‡]	5.3 \pm 2.0 ^e	9.6 \pm 20 ^{ik}
Asian Indian				<20		4.2 \pm 1.5 ^{e,g}	
Caucasian				<20		6.2 \pm 1.6 ^{e,g}	
Banerji <i>et al</i> ⁷							
Asian Indian	20	39	25	33 ^a	12.1 \pm 4.3 [‡]	25.0 \pm 7.8 ^f	6.3 \pm 4.2 ^{jl}
Liew <i>et al</i> ²⁹							
Asian Indian	10	26	22	27 ^c	7.8 \pm 3.0 [§]	9.5 \pm 0.4 ^{e,h}	9.9 \pm 3.3 ⁱ
Caucasian	10	27	23	23 ^c	4.2 \pm 1.4 [§]	12.5 \pm 0.5 ^{e,h}	18.8 \pm 9.2 ⁱ
Chinese	10	25	22	20 ^c	5.4 \pm 2.9 [§]	10.1 \pm 0.4 ^{e,h}	14.1 \pm 3.5 ⁱ

Mean values \pm SD; Reference numbers as in text.

* Comparisons should be made between parameters with same letter superscript since different authors used different methods of calculation. Recalculated values are presented where possible, based on reported data.

Plasma insulin measured by: [†]ELISA, [‡]RIA, [§]Microparticle enzyme immunoassay

Per cent body fat determined by: ^acomputed axial tomography, ^bunder water weighing, ^cbioelectric impedance analysis.

Units and recalculations:

^d mg/kg body weight/min calculated over last 40 min of clamp, ^e mg/kg lean body mass/min calculated over last 40 min of clamp, ^f μ m/kg/min calculated over last 60 min of clamp,

^g [mg/(kg fat free mass/min)], reported for a subgroup with % BF < 20%, no other details reported,

^h mg/kg lean body mass/min calculated over last 40 min of clamp; this value was recalculated from the reported mean Clamp IS and plasma insulin during the clamp, ⁱ [mg/(kg fat free mass/min)]/(μ U/ml) calculated over last 40 min of clamp²¹, ^j [mg/(kg fat free mass/min)]/(μ U/ml) calculated over last 60 min of clamp²¹, ^k recalculated from reported mean glucose disposal rate and plasma insulin over last 40 min of clamp, ^l recalculated from reported mean glucose disposal rate and plasma insulin during clamp over the last 60 min

However, when both the groups were considered separately there was no significant relationship with clamp IS and anthropometric variables which was not surprising, because the range of the independent variables was considerably constrained, and the sample size smaller. In addition to the anthropometric and body composition variables addressed in this study, the effect of abdominal fat distribution and the impact of intra-myocellular lipids on Clamp IS in this population would be important issues to address in future studies. The current study also compared the Clamp IS and glucose metabolism rates of Indians from the present study with those of other ethnic groups and demonstrated that our subjects had largely lower Clamp IS and reduced glucose metabolism rates, although their % BF was not higher than that in the other groups. There were significant correlations

between Clamp IS and other indices of IS, barring HOMA1-IR and QUICKI.

The relationship between BMI, % BF and IS (using HOMA indices) in Indians has been well documented^{2,3,7,10} albeit in subjects across a generally higher BMI range, encompassing overweight and frankly obese subjects with a lower BMI limit of approximately 20 kg/m² (Ref.7). The high degree of correlation between Clamp IS and % BF as well as the differences in Clamp IS between NW and LBMI groups suggests that the relation between BMI and IS is likely to be a continuum with no specific cut-off for BMI beyond which the IS suddenly decreases. The relatively lower IS in individuals with normal BMI may also be due to the conjoint effects of increased body fat and a relatively reduced total body muscle

mass, since studies done on Asians in comparison to Caucasian and African-American (adult and pre-pubertal children) subjects have indicated that Asians have less skeletal muscle mass as a fraction of body weight³⁰. A previous study done between age and BMI matched Indians and Caucasians demonstrated that Indians had more adipose tissue without differences in the distribution of adipose tissue; in addition, there was a reduced lean tissue mass⁹, indicating that factors other than body fat and its location are involved in determining IS. Earlier studies have also demonstrated that the prevalence of IR in healthy, young, lean, migrant Asian-Indian men is 3 to 4 fold greater than lean men of other ethnic groups³¹, and Indians appear to have low IS even at a normal BMI and at relatively lower % BF than Caucasians^{2,3,7,29}. The NW subjects in the present study had lower glucose disposal rates to most of the relatively similar BMI Asian Indians from other studies, with the exception of the study of Chandalia *et al.*³, where those Asian Indians had significantly lower (by about 25%) glucose disposal rates. The latter finding might be related to the fact that the Asian Indian subjects were older and were 'temporarily living' in the United States when they were recruited for that study³. This is significant, since temporary migrants have a greater exposure to nutrition and lifestyle related transitions leading to a greater risk for diabetes. However, the recalculated Clamp IS values were comparable and this discrepant result may have been due to technical differences in methodologies, since that study used a non steady state isotope dilution technique to measure glucose disposal rates, as well as an RIA based method to measure plasma insulin levels³. The different insulin infusion rate used in other studies^{3,7}, will not affect the insulin sensitivity values but changes in the time taken to achieve a steady state plasma insulin levels occur. Conversely, the finding that the present study subjects had significantly lower glucose disposal rates than in other studies is not easily explained; this might relate to other phenotypic variables such as physical activity levels and unrecorded differences in muscle mass or function. In addition, the differences in these comparisons were only about 10 per cent, which could also be due to methodological differences. Therefore, comparisons between studies, particularly for the HEC method, even when performed according to a similar protocol, are fraught with difficulty and can only be indicative. Another significant comparison related to the higher reported Clamp IS in Asian Indians from Singapore²⁹

in relation to the lower values observed in the present study. One interesting possibility is that the former subjects were possibly much more active, with a reported PAL of 1.7, as compared to the present study subjects whose PAL was only 1.4. This is a large difference in physical activity, delineating these groups into what might be considered sedentary and moderately active. In addition, the significant relationship between % BF and Clamp IS, which was evident even at a low BMI in our study, suggests that lifestyle modifications, particularly aimed at improving activity levels and reducing the slope of the % BF to IS relationship, need to be initiated even in relatively low BMI, apparently low risk individuals. This would be important to evaluate in prospective studies and would also address the issue of the extent to which the lower IS of Indians is lifestyle related and modifiable, as opposed to being genetic in origin.

According to the American Diabetes Association Consensus Development Conference on insulin resistance, the HEC and minimal model method applied to the frequently sample intravenous glucose tolerance test (FSIVGTT) are the only two methods that satisfactorily assess peripheral insulin resistance³². There are several other convenient methods to determine IS besides the HEC method. Matthews *et al.*¹¹ proposed the HOMA1-IR model to predict insulin sensitivity and β -cell function from fasting plasma insulin and glucose concentrations. HOMA1-IR and IS from the HEC have been shown to be highly correlated^{11,12}, with correlation coefficients between 0.85 to 0.88. The subjects studied in these reports were elderly individuals, with high BMI and fasting insulin levels, when compared to the present study subjects. The absence of a correlation between HOMA1-IR and Clamp IS in the present study may be because of different subject characteristics; the present study subjects were younger with a smaller age range, had lower fasting plasma insulin levels and a smaller range of HOMA1-IR than the earlier studies^{11,12}. The coefficient of variation (CV) for HOMA1-IR was earlier reported to be 31 per cent¹¹ but is now reduced to 9.4 and 7.8 per cent¹² in nondiabetic and diabetic subjects.

While there were significant correlations between Clamp IS and HOMA2 model indices in the present study and while it may be that the more convenient indices based on fasting plasma glucose and insulin values have uses in epidemiological studies, these

indices are clearly not equivalent and depend on the type of subjects being studied. Clamp IS measured by the HEC is clearly a more discriminatory and sensitive marker, as it shows differences between NW and LBMI groups while the other indices do not. While the use of simpler indices has provided much information in epidemiological studies in India, the wider adoption of IS with the HEC will allow for subtle pathophysiological processes affecting IS to be studied and will allow the mechanisms, such as those related to physical activity, muscle mass and function, that contribute to the apparently greater propensity for diabetes in Indians, to be carefully delineated.

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