

REPLICATION OF *IGF2-INS-TH**5 HAPLOTYPE EFFECT ON OBESITY AND STUDY OF RELATED PHENOTYPES IN OLDER MEN

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ABSTRACT

Inter-individual variation of the *IGF2-INS-TH* region influences risk of a variety of diseases and complex traits. A previous study [1] identified a haplotype (designated *IGF2-INS-TH**5 and tagged by allele A of *IGF2* *Apal*, allele 9 of *THO1* and class I alleles of *INS* VNTR) associated with low body mass index (BMI) in a cohort of UK men (Northwick Park Heart Study II). We conducted specific tests of *5 haplotype (versus non-*5 haplotypes) in a different cohort of men (East Hertfordshire) characterised in more phenotypic detail. We observed replication of weight effect for the *5 haplotype: significant associations with lower BMI (-1.81kg/m², P = 0.0045), lower waist circumference (-6.3cm, P = 0.001) and lower waist-hip ratio (-5%, P = 0.000). This haplotype also marks nearly two-fold lower 120 minute insulin (P = 0.004) as well as low baseline insulin (-11.02pmol/l, P = 0.043) and low 30 minute insulin (-64.44pmol/l, P = 0.072) in a glucose tolerance test. Our results, taken together with other data on IGFII levels and TH activity, point to the importance of *5 as an integrated polygenic haplotype relevant to obesity.

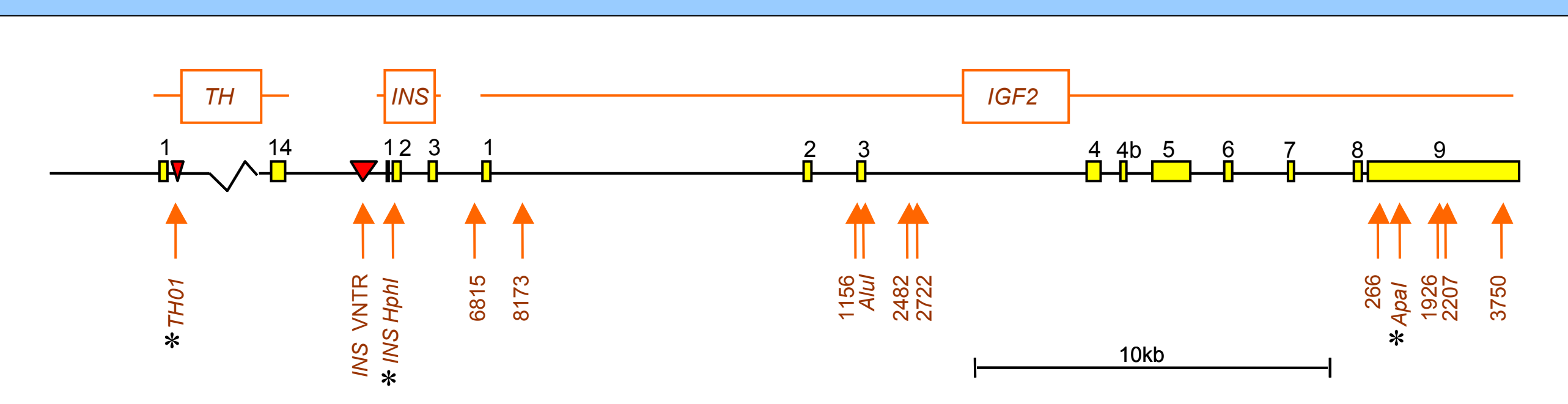


Figure 1. Genetic map of the *IGF2-INS-TH* region spanning around 40 kb of 11p15.

Exons of *TH*, *INS* and *IGF2* are numbered and represented by boxes. Indicated by an asterisk are the three markers analysed in this work (*IGF2* *Apal*, *INS* *HphI* and *THO1*). We have previously shown that the resulting haplotypes from these three markers tag the commonest haplotypes of the 14 polymorphisms shown in this figure, giving haplotypic analyses with an increased power (due to fewer missing data) and a reduced risk of false positive findings related to low-frequency haplotypes [1].

MATERIAL AND METHODS

The identification of the *5 haplotype was done as previously described [1]. In brief, we used the PHASE program version 2.0 [2] in order to determine, with a predefined level of confidence set at $\geq 90\%$, the two haplotypes resulting from three polymorphisms (*IGF2* *Apal*, *INS* *HphI* and *THO1*) for each one of the individuals analysed. The three polymorphisms were genotyped as previously described ([1] and references therein) from a sample of 131 unrelated UK Caucasian men from the East Hertfordshire cohort. These subjects were selected from among all births in the county of Hertfordshire UK during 1920-1930, who were followed forward and found to be alive and still resident there in 1990 to 1995. The subset selected for detailed evaluation of obesity-related phenotypes comprised those willing to undergo an oral glucose tolerance test (GTT), they did not differ significantly from the larger group in regard to birthweight or socioeconomic status. The 19 traits analysed were: birthweight, weight at 1 year, weight, height, BMI, waist circumference (WC), hip circumference, waist-hip ratio (WHR), plasma triglycerides, systolic blood pressure, diastolic blood pressure, fasting plasma concentrations of proinsulin and traits related to oral GTT: plasma concentrations of insulin and glucose at baseline, at 30 and at 120 minutes after an oral load of 75g of glucose, and insulin area under the curve during the GTT. Given prior hypothesis suggesting that *5 haplotype has an effect on weight [1], we compared, for each trait, the effect of this haplotype with the effect of the remaining haplotypes pooled together (defined here as not*5). The differences in the mean values observed for each trait between *5 and not*5, were compared using the Student's *t* test. Adjustments for covariates were also performed using multiple regression analyses. The statistical analyses were conducted using SPSS (Windows ver. 10).

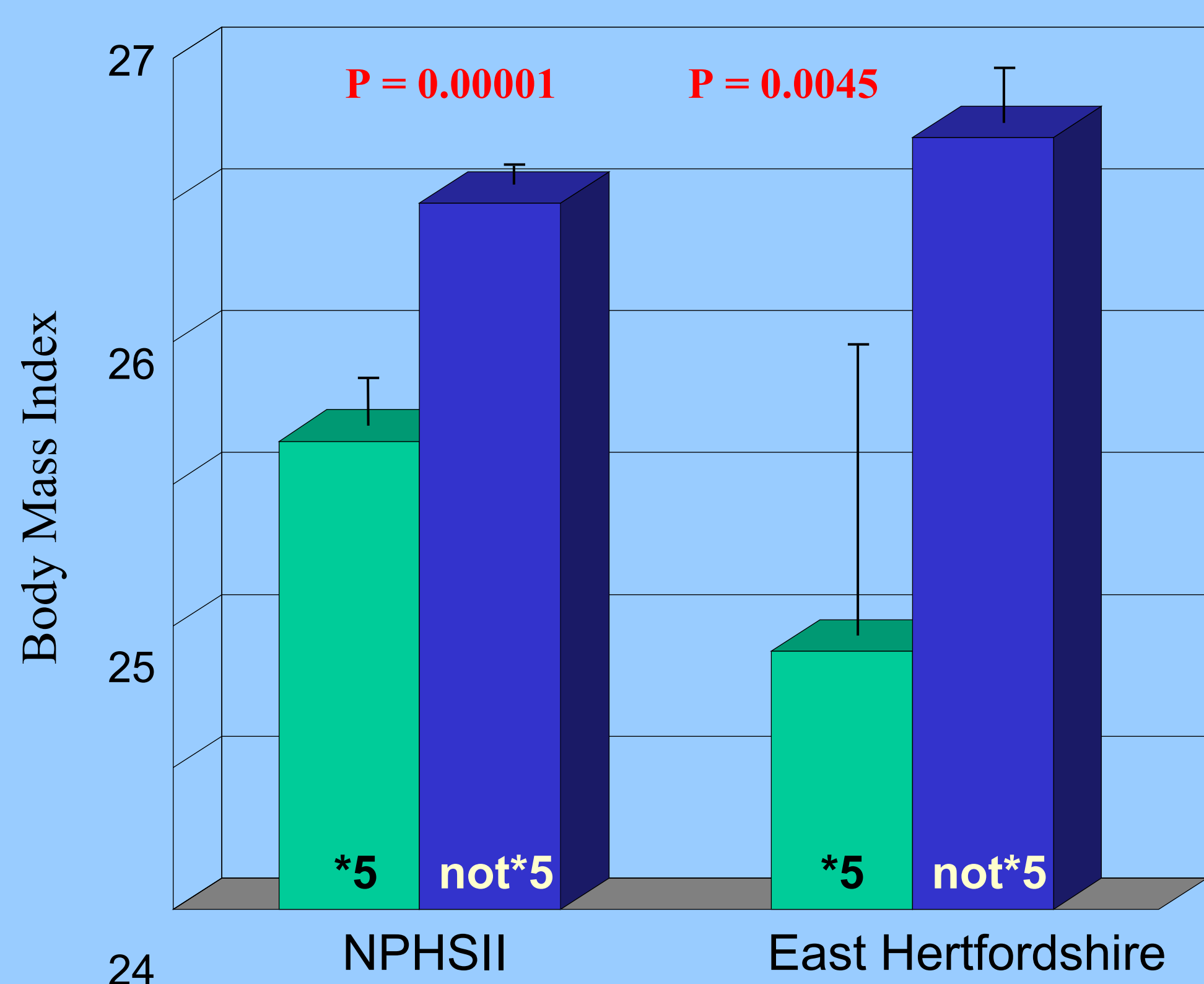


Figure 2. Mean BMI observed in NPHSII [1] and in East Hertfordshire for *5 and for the pool of remaining haplotypes (not*5).

In accordance with previous evidence [1], haplotype *5 was found to show a significantly lower BMI when compared with not*5 (P = 0.0045) in East Hertfordshire. There is 1.81 unit BMI difference, whereas in the NPHSII study, *5 group averaged 0.84 units lighter than not*5 group (P = 0.00001). Error bars represent S.E. of the mean.

Table 1. Effect of the *IGF2-INS-TH5 haplotype on metabolic and cardiovascular risk traits.**

The mean values, standard errors of the mean and number of haplotypes observed both for *IGF2-INS-TH**5 and for the pool of remaining haplotypes are shown. P₁ and R² are, respectively, the P values and the percentage of total variance explained by *IGF2-INS-TH**5 for each trait, observed in the regression analyses without adjustments for covariates. P₂ and P₃ are the P values observed in multiple regression analyses after adjusting for covariates as listed in the table footnotes.

Phenotype	*5 haplotype			Not*5 haplotypes			P ₁	R ² (%)	P ₂	P ₃
	Mean	S.E. ^a	N	Mean	S.E. ^a	N				
Birthweight (ounces)	129.18 ± 3.03		22	126.97 ± 1.39		240	0.637			
Weight 1yr (ounces)	365.23 ± 5.52		22	366.12 ± 2.82		240	0.925			
Weight (kg)	74.39 ± 3.10		22	79.38 ± 0.64		240	0.015	1.8	0.019^b	0.031^c
Height (m)	1.73 ± 0.01		22	1.72 ± 0.00		240	0.722			
BMI (kg/m ²)	24.91 ± 1.01		22	26.72 ± 0.19		240	0.005	2.6	0.006^b	0.009^c
Waist circumf. (cm)	91.40 ± 2.85		22	97.66 ± 0.53		240	0.001	3.9	0.002^b	0.003^c
Hip circumf. (cm)	101.98 ± 1.64		22	104.26 ± 0.41		240	0.112			
Waist:Hip (proportion)	0.89 ± 0.01		22	0.94 ± 0.00		240	0.000	5.9	0.000^b	0.000^c
Systolic BP (mm Hg)	157.68 ± 5.64		22	163.12 ± 1.44		240	0.281			
Diastolic BP (mm Hg)	88.14 ± 2.84		22	90.10 ± 0.69		240	0.418			
Plasma TG (mmol/l)	1.42	1.11	22	1.48	1.03	236	0.688			
Proinsulin (pmol/l)	1.99	1.14	21	2.94	1.05	233	0.015	2.3	0.131 ^d	0.473 ^e
Insulin 0 (pmol/l)	31.50	1.12	20	42.52	1.04	232	0.043	1.6	0.313 ^d	0.680 ^e
Insulin 30 (pmol/l)	217.02	1.09	22	281.46	1.04	228	<u>0.072</u>	1.3	0.241 ^d	0.289 ^e
Insulin 120 (pmol/l)	87.36	1.20	21	151.41	1.06	223	0.004	3.3	0.021^d	<u>0.095^e</u>
Insulin AUC OGTT	18583	1	19	26370	1	215	0.015	2.5	<u>0.086^d</u>	0.186 ^e
Glucose 0 (mmol/l)	5.64	1.03	22	6.05	1.01	236	0.056	1.4	0.300 ^d	0.431 ^e
Glucose 30 (mmol/l)	8.67	1.04	22	9.49	1.01	236	0.030	1.8	0.271 ^d	0.621 ^e
Glucose 120 (mmol/l)	5.81	1.05	21	6.69	1.02	235	<u>0.076</u>	1.2	0.297 ^d	0.500 ^e

^a Means and S.E. for plasma TG, proinsulin, insulin 0, insulin 30, insulin 120, insulin AUC OGTT, glucose 0, glucose 30 and glucose 120 are geometric, so that the geometric S.E. are multiples of the relevant means; ^b Adjusted for age; ^c Adjusted for age and smoking; ^d Adjusted for Waist:hip ratio; ^e Adjusted for Waist:hip ratio, age and smoking.

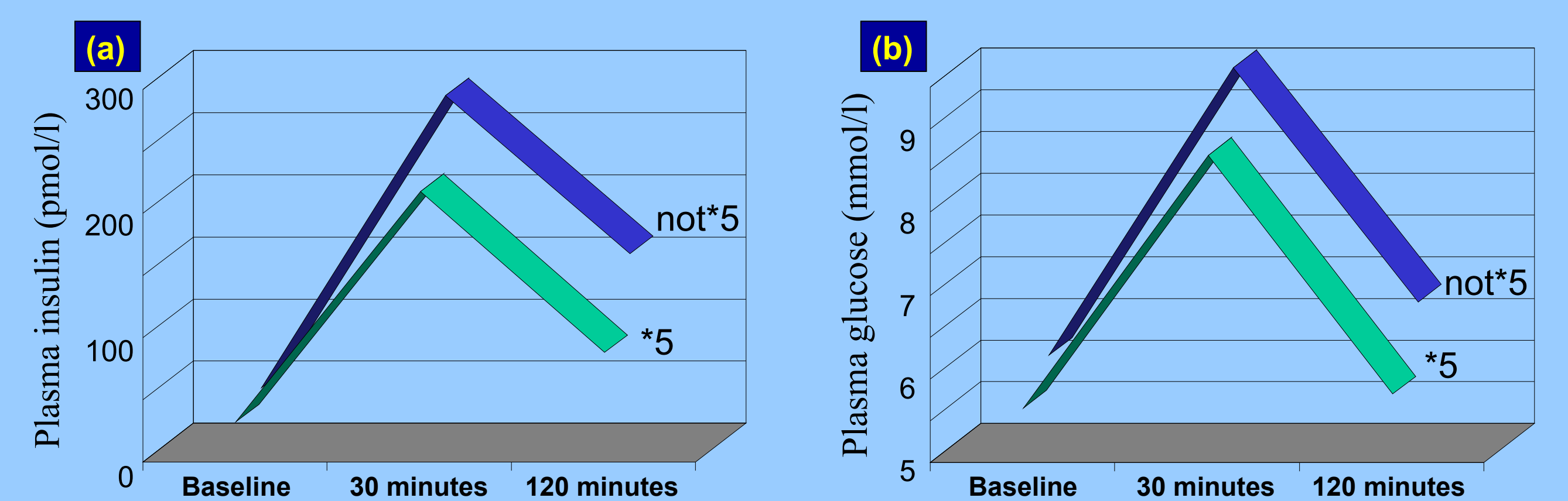


Figure 3. Mean plasma insulin levels (a) and mean plasma glucose levels (b) observed for *5 and for not*5 haplotypes at baseline and at 30min and 120min after an oral load of 75g of glucose.

Lower plasma levels for both insulin and glucose were associated with *5 both at baseline and after an oral GTT.

CONCLUSIONS

- This study both replicates evidence of the protective effect of *IGF2-INS-TH**5 haplotype against obesity in men and extends detail on a range of relevant subphenotypes.
- The finding presented here that WC and WHR are significantly lower for *5 suggests that the reduction in BMI conferred by this haplotype occurs mainly via reduction of visceral obesity.
- There is evidence in the literature involving all three genes, *IGF2*, *INS* and *TH* in the regulation of body weight, so the functional element(s) of *5 which determine the weight effect could reside in the any of these genes.
- Given that insulin is an inhibitor free fatty acid mobilization [3] and a decline in insulin secretion stimulates free fatty acid release from the adipose tissue [4], a low expressor haplotype of insulin could be a primary determinant of lower weight. However, as an increase of insulin secretion in proportion to accumulated fat counteracts insulin resistance and protects individuals from hyperglycemia [5], it is also possible that the lower insulin levels after an oral GTT found for *5 could be secondary to the lesser insulin resistance of lean individuals.
- It is known that visceral fat is more lipolytic in response to catecholamine stimulation than is subcutaneous fat [6]. Also known is that the *THO1* microsatellite has a role in the transcription of this gene, alleles 9 and 9.3 stimulating TH expression [7]. The lower visceral fat associated with *5 could therefore reflect an increased lipolysis of visceral fat stimulated by catecholamines via a comparatively increased expression of *TH* conferred by the *5 constituent *THO1* allele 9.
- It has been suggested that the expression of an *Igf2* transgene in adult skin reduces the amount of body fat [8], whereas low plasma IGFII concentrations predict weight gain and obesity [9]. Homozygotes for allele A at *IGF2* *Apal* site in the 3' noncoding region show a mean body weight 3.3kg lighter and show higher mean serum IGFII levels than the common (GG) homozygotes [10]. It is therefore plausible that *5 predicts high IGFII levels and that this high expressor status is a primary protection against obesity.

References

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