

Fat Mass and Obesity Associated Gene Variants Are Associated With Increased Growth Hormone Levels and Affect Glucose and Lipid Metabolism in Lean Women

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Summary

First intron variability of the fat mass and obesity associated gene (*FTO*) has strong impact on adiposity. We focused on lean women carrying the most "obesity-risk" haplotype to study their anthropometric parameters and hormonal and metabolic profile. Genotype-phenotype correlation was performed in a group of 172 lean women (body mass index (BMI) ≥ 18.5 and < 25 kg/m²; age 26.8 ± 7.26 years), 77 of them used hormonal contraceptives. Even in lean women the association of the risk haplotype CAGA with BMI was confirmed but it did not influence the anthropometric indices of body composition. CAGA carriers compared to non-carriers had significantly higher both fasting ($p=0.016$) and post glucose load ($p<0.001$) levels of growth hormone (GH), significantly higher glucose, insulin and C-peptide levels in the late phase of oGTT and lower fasting concentration of total cholesterol and LDL-cholesterol. Administration of hormonal contraceptives further increased observed hormonal and metabolic effects in CAGA carriers. We conclude that higher levels of GH in lean women carrying the *FTO* "obesity risk" haplotype could protect them from the development of obesity. The relation between the *FTO* gene variability and GH secretion has to be elucidated. This is the first study demonstrating the interaction of *FTO* genotype with hormonal contraception.

Key words

Fat mass and obesity associated gene • Gene variants • Glucose metabolism • Growth hormone • Hormonal contraception

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Introduction

Genome-wide association studies have led to the identification of a number of candidate genes related to worldwide diseases such as type 2 diabetes mellitus or obesity and polycystic ovary syndrome. In 2007, the fat mass and obesity associated gene (*FTO*) was identified as a gene with strong obesity-related traits. Several independent laboratories detected a range of single nucleotide polymorphisms (SNPs) in the first intron of the gene with a great impact on adiposity (Dina *et al.* 2007, Frayling *et al.* 2007, Scuteri *et al.* 2007). Consequently, the association of the *FTO* gene with obesity was confirmed in various populations around the world. Through its association with obesity, *FTO* was also confirmed as a susceptibility gene for diabetes mellitus type 2 (DM2) (Frayling *et al.* 2007, Scott *et al.* 2007) and polycystic ovary syndrome (PCOS) (Attaoua *et al.* 2008, Barber *et al.* 2008). However, the effects of these intronic variants on *FTO* function are still unclear.

FTO is a member of the AlkB-related non-heme iron- and 2-oxoglutarate-dependent dioxygenase family. Studies in mice suggest that it may be involved in nucleic acid demethylation (Gerken *et al.* 2007, Sanchez-Pulido and Andrade-Navarro 2007) and that it functions as a transcriptional coactivator playing a role in the epigenetic regulation of the development and maintenance of fat tissue (Gerken *et al.* 2007).

The *FTO* gene (16q12.2) is widely expressed in a variety of human tissues, with the highest levels in the brain, particularly in hypothalamic nuclei, a

well-defined key regulatory centre of energy homeostasis (Dina *et al.* 2007, Frayling *et al.* 2007). Its mRNA level is regulated by the energy balance in response to feeding or fasting (Gerken *et al.* 2007, Frederiksson *et al.* 2008). The association of *FTO* gene variants with cerebrocortical insulin resistance typical for obese humans was documented as well (Tschritter *et al.* 2007).

FTO is also expressed in pancreatic islets, skeletal muscle, in liver as well as in adipose tissue (Dina *et al.* 2007, Grunnet *et al.* 2009). The expression of *FTO* in adipose tissue was usually higher in obese individuals. The role of *FTO* in peripheral fat lipolysis by which the gene could affect the body weight regulation was also reported (Wåhlén *et al.* 2008). The study of skeletal muscle biopsies revealed increased energy efficiency and potentially increased mitochondrial coupling in risk allele (rs9939609, intron 1) carriers vs. non-carriers which may contribute to the increased risk of obesity and DM2 (Grunnet *et al.* 2009). However, *FTO* genotype did not influence the mRNA expression either in skeletal muscle or in adipose tissue (Wåhlén *et al.* 2008, Grunnet *et al.* 2009).

In our previous study which included 1388 Czech adults of Caucasian origin an association of 4 SNPs in the 1st intron of *FTO* gene with BMI ($p < 0.001$), waist circumference ($p < 0.001$) as well as with leptin level ($p = 0.003$) and glucose levels at the 60th, 90th and 120th min of the oGTT ($p < 0.005$) was confirmed (Vcelak *et al.* 2008). In this study we focused on a group of lean healthy control women to evaluate the associations of the most risk haplotype CAGA with anthropometric data, parameters of glucose and lipid metabolism, selected hormones and adipokines. We also studied the possible role of interaction of the genotype with hormonal contraception which has been underestimated until now.

Materials and Methods

Subjects characteristics

Our study cohort consisted of 172 lean ($BMI \geq 18.5$ and $< 25 \text{ kg/m}^2$) healthy women carrying combinations of two major *FTO* haplotypes TGTT and CAGA ($BMI 21.5 \pm 1.99 \text{ kg/m}^2$; age 26.8 ± 7.26 years). Subjects with family history of DM2, gestational diabetes, PCOS and impaired fasting glucose or impaired glucose tolerance were excluded from the study. Seventy-seven women used hormonal

contraceptives (HC): 52 % women used low-dose oral contraceptives (OC; ethinylestradiol (EE) or estradiol-valerate (E2V) below 30 ug); 42 % combined OC (with 30-35 ug EE and third-generation progestogens) and the remaining 6 % contraceptive coil.

The protocol of the study was approved by the Ethic Committee of the Institute of Endocrinology and all subjects gave their informed consent.

Anthropometric measurements

Body weight, height, waist and hip circumference were measured in all subjects, body mass index (BMI) and waist-to-hip ratio (WHR) were calculated. To reflect the percentage of body fat we used body adiposity index (BAI, $BAI = [\text{hip circumference}/\text{height}^{1.5}] - 18$ [%]) (Bergman *et al.* 2011).

Metabolic and hormonal characteristics

The baseline levels of glucose, C-peptide, immunoreactive insulin, total cholesterol, high-density lipoprotein (HDL-) and low-density lipoprotein (LDL-) cholesterol, triglycerides and glycosylated hemoglobin (HbA_{1c}) [Cobas Integra, Roche Diagnostics], proinsulin [ELISA, DRG Diagnostics, Germany], glucagon [RIA, IBL], growth hormone (GH) and insulin-like growth factor 1 (IGF-1) [IRMA, Immunotech], plasma renin activity (PRA) [RIA, Immunotech] and leptin [RIA, LINCO research] were assessed. Free fatty acids concentrations and their composition were measured by HPLC. All women underwent 3-h oral glucose tolerance test (oGTT) with 75 g glucose dose; during the test levels of glucose, C-peptide, insulin (in 30 min intervals), proinsulin, glucagon, free fatty acids (at 0, 60th, 180th min) and growth hormone (at 0, 60th, 120th min) were determined. Areas under the curves for glucose, C-peptide and insulin were calculated (AUC). To assess insulin sensitivity and beta-cell function, homeostasis models assessments (HOMA R and HOMA F, Matsuda index, Cederholm index and insulinogenic index) were used and derived disposition indices (beta-cell function*insulin sensitivity) were calculated (Matthews *et al.* 1985, Cederholm and Wibell 1990, Matsuda and DeFronzo 1999).

Genotyping

DNA was extracted from peripheral leukocytes using the commercial kit (QIAamp DNA Blood Kit, QIAGEN, Germany). The four intronic single nucleotide polymorphisms (SNPs), rs1421085 (T/C),

rs1121980 (G/A), rs17817449 (T/G) and rs9939609 (T/A) in the *FTO* gene were assessed by ABI TaqMan SNP Genotyping Assays (LightCycler 480 System, Roche). Haplotype combinations were generated using a programme PHASE version 2.1. (<http://stephenslab.uchicago.edu/software.html>).

Statistical analyses

For statistical evaluation NCSS 2004 (Statistical Solutions, Saugus, USA) software was used. Data are given as means \pm SDs and as percentages. Data in Figures are shown as medians. The Chi-square test was used to assess deviation from Hardy-Weinberg equilibrium of the genotypic frequencies by calculating expected frequencies of genotype. Differences in biochemical and anthropometric data between subgroups were tested by non-parametric Mann-Whitney test, the two tailed *p* values <0.05 were considered to be significant.

Results

FTO gene haplotypes

The component obesity risk SNPs: rs142108 (T/C); rs1121980 (G/A); rs17817449 (T/G) and rs9939609 (T/A) are located within 19.6 kb region in intron 1 of the *FTO* gene and they are in linkage disequilibrium, however, programme PHASE generated 10 haplotype combinations in the cohort of our pilot study. For this study we selected 172 lean women – carriers of two major haplotypes TGTT and CAGA – 54 (31.4 %) were TGTT homozygotes; 78 were TGTT/CAGA heterozygotes (45.3 %) and 40 (23.3 %) were CAGA homozygotes.

Anthropometric and metabolic parameters in carriers and non-carriers of the FTO risk haplotype

Anthropometric and hormonal parameters were compared in carriers of the most risk haplotype CAGA (CAGA/CAGA homozygotes together with TGTT/CAGA heterozygotes) vs. CAGA non-carriers (TGTT homozygotes). Even in a subgroup of 172 lean women the association of the CAGA risk haplotype carriership with a higher BMI was apparent (CAGA carriers 21.7 ± 1.98 kg/m² vs. non-carriers 21.1 ± 1.94 kg/m²; *p*=0.038). However, the CAGA carriers vs. non-carriers did not differ in waist circumference, WHR and percentage of body fat.

We demonstrated that the CAGA-carriers had

significantly higher levels of glucose, insulin and C-peptide in the late phase of the oGTT in comparison with CAGA non-carriers. The CAGA carriers had a tendency to increased proinsulin levels at 60th and 180th min of the oGTT. CAGA carriership was also associated with higher growth hormone levels in the fasting state and at 60th min of the oGTT (Fig. 1). CAGA carriers had significantly lower levels of total cholesterol (4.2 ± 0.81 vs. 4.5 ± 0.75 mmol/l, *p*=0.030) and LDL-cholesterol (2.2 ± 0.71 vs. 2.4 ± 0.69 mmol/l, *p*=0.011). There were no differences in IGF-1, PRA, glucagon, leptin, HDL-cholesterol, free fatty acids concentrations and their composition. Lean women with and without CAGA haplotype did not differ in beta-cell function (HOMA F, insulinogenic index), insulin sensitivity (HOMA R, Matsuda index, Cederholm index) and disposition indices derived from oGTT (data not shown).

Anthropometric and metabolic parameters with respect to HC administration

In our cohort of women, 77 of them were using the hormonal contraceptives (HC). There was nonsignificant effect of HC itself on anthropometric parameters. We assume that HC usage had similar influence on metabolic parameters as risk haplotype carriership (Fig. 2). Women with HC (without respect to *FTO* haplotype – the distribution of CAGA carriers was similar in subgroups of HC users and non-users; chi-square test, NS) had higher levels of glucose, insulin and C-peptide in the late phase of the oGTT and had also higher GH levels in response to glucose load. In addition, women with HC had significantly higher levels of proinsulin in all phases of the oGTT compared to HC non-users. Regarding the other tested parameters, the HC usage was also associated with increased triglycerides (1.05 ± 0.374 vs. 0.75 ± 0.328 mmol/l, *p*<0.001) and increased PRA (0.94 ± 0.174 vs. 0.64 ± 0.076 ng/ml*h, *p*=0.016). The HC influenced neither the insulin secretion nor the insulin sensitivity indices except of Cederholm index which was significantly lower in women using HC in comparison with non-users (81.3 ± 18.15 vs. 86.4 ± 19.89 kg*/mIU, *p*=0.045). In spite of a demonstrated impact of HC on GH levels, there was no difference in IGF-1 levels between HC users and non-users.

The role played by a different composition of contraceptives has not been studied.

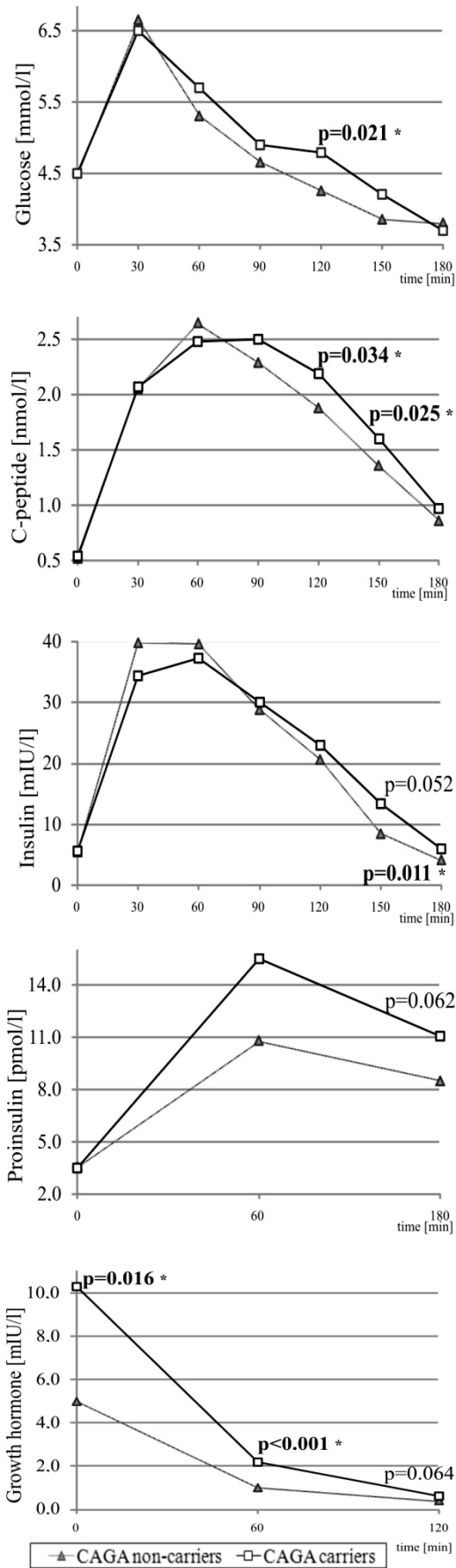


Fig. 1. Metabolic parameters during oGTT in lean women with respect to *FTO* gene CAGA carrier status.

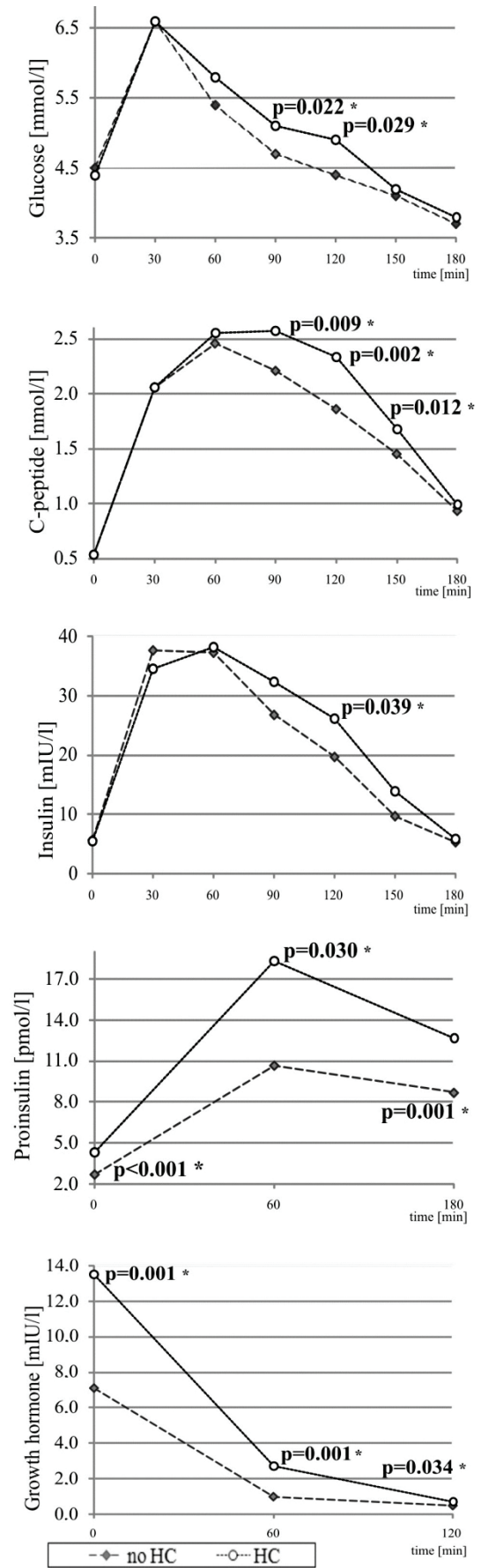


Fig. 2. Metabolic parameters during oGTT in lean women with respect to hormonal contraception (HC) usage.

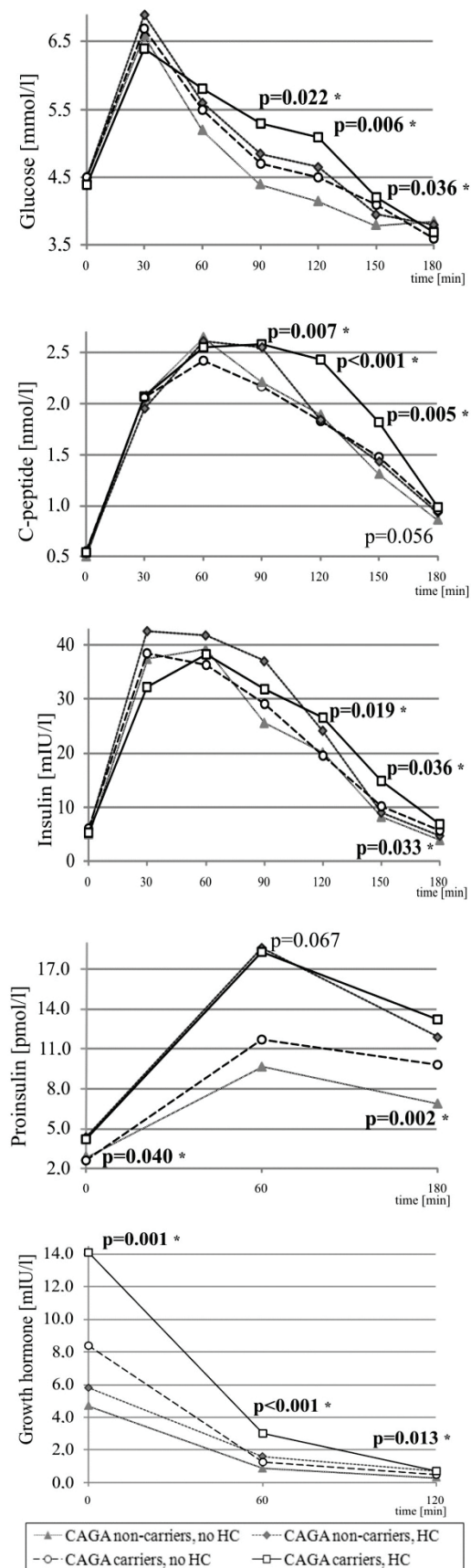


Fig. 3. Metabolic parameters during oGTT in lean women with respect to CAGA carriership and hormonal contraception usage (* statistical significance between CAGA non-carriers without HC and CAGA carriers with HC).

Anthropometric and metabolic parameters with respect to FTO gene CAGA carriership and HC usage

The interaction of the risk FTO haplotype carriership and hormonal contraception is shown in Figure 3. The CAGA carriers using HC had the highest blood glucose, insulin and C-peptide levels in the late phase of oGTT. They also have the highest fasting as well as post glucose load levels of proinsulin and GH in comparison with the other groups, especially with CAGA non-carriers without HC.

When we assessed the effect of HC usage in risk-haplotype carriers (Fig. 3, these p levels are not shown there): the HC users had higher level of glucose at 90th and 120th min (NS, $p=0.030$), C-peptide at 90th, 120th and 150th min ($p=0.040$; 0.001 and 0.004) and proinsulin in all phases of the oGTT ($p=0.008$, NS and 0.041) than HC non-users. Similarly, levels of GH were increased in HC users in all phases of the oGTT ($p=0.002$ and 0.010, NS). Nevertheless, no significant effect of HC was apparent in the subgroup of CAGA non-carriers.

There seems to be an additive effect of risk-haplotype carriership and HC usage on these metabolic parameters.

Discussion

Though there have been many published studies that uniformly document the association of FTO gene variants in intron 1 with obesity, the mechanisms by which these variants affect FTO function and how the FTO gene itself contributes to the development of obesity are still not well understood.

The following questions were raised in our study: 1) what are the anthropometric and metabolic consequences of CAGA carriership in lean women, 2) could they be influenced by hormonal contraception and 3) how are lean women carrying the obesity risk FTO haplotype protected from obesity?

Association of the risk haplotype with BMI was detected but not with other anthropometric measurements. It confirms the globally observed association of variants in the first intron of FTO gene with increased BMI, which was also reported in the Czech general population (Vcelak *et al.* 2008, Hubacek *et al.* 2008, 2009).

In previously published studies genetic variations in the FTO gene were associated not only with increased body weight, but also with other metabolic traits like increased insulin secretion, leptin levels,

reduced insulin sensitivity and impaired lipid profile. However, these influences on metabolic traits mostly lost their statistical significance after adjustment for the independent effects of obesity (Fall *et al.* 2013). Our results indicated that even in lean women, the risk CAGA haplotype was associated with increased stimulated glucose, insulin and C-peptide during the late phase of oGTT. Interestingly, CAGA carriership was strongly associated with higher levels of growth hormone (GH) both in the fasting state and in response to glucose load. This is a novel finding regarding metabolic associations of *FTO* variants. However, Roskopf *et al.* (2011) proposed that the GH/IGF-1 axis could be a mediator for the relationship between *FTO* and BMI.

In fact, increased GH levels could be responsible for the leanness of the studied women carrying the obesity risk CAGA haplotype as well as for the detected metabolic effects found in these women.

GH is synthesized and secreted by somatotrophic cells in the anterior pituitary. It plays a key role in the control of several complex physiological processes, including growth and metabolism. GH stimulates protein anabolism, enhances fat utilization by enhancing triglyceride breakdown and oxidation in adipocytes. GH has anti-insulin activity; it suppresses the glucose uptake in peripheral tissues and enhances glucose synthesis in the liver. In human obesity, the GH/IGF-1 axis is altered at different levels. Increased adiposity is characterized by blunted GH secretion which is coupled with low, normal or high serum IGF-1 levels (Savastano *et al.* 2014, Cordoba-Chacon *et al.* 2015, Pena-Bello *et al.* 2015). GH fragments as well as the GH-releasing hormone analogue were suggested as possible anti-obesity agents (Kokshoorn *et al.* 2011, Berryman *et al.* 2013).

We suggest that the elevated beta-cell secretion in the late phase of the oGTT as well as the decreased total- and LDL-cholesterol found in our lean CAGA carriers could be a consequence of increased GH levels in these women (Rudling and Angelin 2001, Weltman *et al.* 2003, Freathy *et al.* 2008, Kokshoorn *et al.* 2011). It could be speculated that the increased GH levels protect women carrying the obesity risk *FTO* haplotype from the development of obesity (Pena-Bello *et al.* 2015). The reason of the elevated GH levels in these women is not known. The IGF-1 levels were not elevated in CAGA carriers. The cause of the dissociation of GH/IGF-1 axis is unclear. It could arise from impaired hepatic IGF-1 production (Ho *et al.* 2003), the role of IGF binding

proteins has also to be considered (Ruan and Lai *et al.* 2010).

A similar finding was obtained in women using hormonal contraception (HC) *per se*. HC users had elevated GH levels compared to HC non-users but these two groups did not differ in the IGF-1 levels. It has already been published that hormonal contraceptives can modulate the GH/IGF-1-axis by reducing IGF-1 levels and increasing diurnal integrated mean GH plasma concentrations (Balogh *et al.* 2000, Ho *et al.* 2003). In our study, the HC users did not differ in anthropometric measurements from non-users. In agreement with published findings (Wynn and Doar 1996, Wynn *et al.* 1996), they had higher levels of triglycerides as well as increased fasting and oGTT stimulated proinsulin and higher late phase glucose, insulin and C-peptide levels, together with modestly impaired insulin sensitivity. Our HC users had increased PRA but without consequent influence on blood pressure.

This study demonstrated for the first time the interaction of *FTO* genotype with hormonal contraception. HC usage potentiated the influence of CAGA carriership on the metabolic parameters or vice versa (Fig. 3). Our data suggest that the response of proinsulin to glucose load is probably more influenced by HC than by *FTO* genotype in contrary to the fasting GH levels where the influence of *FTO* genotype seems to be more determining than HC usage (Fig. 3).

In conclusion, we showed an association of *FTO* risk haplotype CAGA with increased GH levels in lean women which could protect them from obesity. Increased levels of blood glucose, insulin and C-peptide in the late phase of oGTT as well as lower cholesterol and LDL-cholesterol could be a metabolic consequence of elevated GH levels. The HC usage *per se* induced similar metabolic effects and the interaction of CAGA haplotype with HC augmented the impact upon observed biochemical parameters.

The cause of elevated GH levels in lean CAGA carriers as well as a possible role of the *FTO* gene in the complex regulatory network of hypothalamic GH secretion should be further elucidated.

Conflict of Interest

There is no conflict of interest.

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Abbreviations

BAI, body adiposity index; BMI, body mass index; DM2, diabetes mellitus type 2; E2V, estradiol-valerate; EE,

ethinylestradiol; FTO, fat mass and obesity associated gene; GH, growth hormone; HDL, high-density lipoprotein; HC, hormonal contraceptives; IGF-1, insulin-like growth factor 1; LDL, low-density lipoprotein; OC, oral contraceptives; oGTT, oral glucose tolerance test; PRA, plasma renin activity; PCOS, polycystic ovary syndrome; SNPs, single nucleotide polymorphisms; WHR, waist to hip ratio.

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