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Can genetic-based advice help you lose weight? Findings from the Food4Me European randomized controlled trial[1,2]

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Key Words: *FTO*, genotype, weight, personalized nutrition, randomized controlled trial

ABSTRACT

Background

There is limited evidence on whether genotype-tailored advice provides extra benefits in reducing obesity-related traits than conventional one-size fit all advice.

Objective

The objective was to determine if disclosing information on *FTO* genotype risk had a bigger effect on reduction of obesity-related traits in risk carriers than non-risk carriers across different levels of personalized nutrition.

Design

683 participants (51% women; age range 18-73 y, body mass index ≥25.0 or waist circumference (WC) >88cm and >102 cm for women and men) from the Food4Me randomized controlled trial were included in this analysis. Participants were randomized to four interventions arms (Level 0: Control group, Level 1: "Dietary" group; Level 2: "Phenotype" (BMI, WC, metabolic markers) group, Level 3: "Genetic" group). *FTO* (SNP rs9939609) was genotyped at baseline in all participants but only those randomized to Level 3 were informed about their genotype. Level 3 participants were stratified into risk (AA/AT) and non-risk carriers (TT) of the *FTO* gene for these analyses. Height, weight and WC were self-measured and reported at baseline, months 3 and 6.

Results

Changes in adiposity markers were larger in participants who were informed that they carried the *FTO* risk allele (Level 3 AT/AA carriers, n=139) compared with the non-personalized group (Level 0, n=171), but not compared with the other personalized groups (Level 1, n=153 and Level 2, n=173). Reductions in weight and WC at month 6 were greater for *FTO* risk carriers (BMI, n= 139 and WC, n=71) compared with non-carriers (BMI, n=47 and WC, n=27) in Level 3 (-2.28 kg

[-3.06, -1.48] vs. -1.99 kg [-2.19, -0.19], *P*=0.037; and -4.34 cm [-5.63, -3.08] vs. -1.99 cm, [-4.04, -0.05] *P*=0.048, for weight and WC, respectively).

Conclusions

Larger body weight and WC reductions were observed for risk carriers compared with non-risk carriers of the *FTO* gene. However, adding genotypic information to the tailored feedback did not enhance the effect of intervention compared with personalization based on diet or diet and phenotype alone.

INTRODUCTION

Over the past 30 years the prevalence of obesity has increased markedly with 17% of European adults(1) and 9% of adults globally now being obese (2). Obesity is a major risk factor for non-communicable diseases (NCDs) including type 2 diabetes (T2D), cardiovascular diseases (CVDs) and many cancers (3, 4). This emphasizes the importance of initiatives aimed at changing lifestyle to prevent and to reduce excess body weight (5). Although previous intervention strategies have mainly focused on "one size fits all" approaches to change dietary and PA behaviors, recent studies have used personalized approaches, e.g. tailored web-based interventions (6-10). There is mixed evidence about the effect of personalized interventions compared with conventional interventions in achieving behavioral changes, but results for weight loss seem promising (11-14).

Reductions in cost and time needed for genome sequencing and enhanced ability to extract relevant information, e.g. disease risk, have fuelled interest in use of personal genetics to tailor interventions (15, 16). However, the effectiveness of genetic-based information in facilitating behavior change is unclear. A recent systematic review called for more, and larger, randomized controlled trials (RCTs) to determine whether DNA-based advice motivates people to make appropriate behavioral changes (17).

Variants in the first intron of the fat mass and obesity associated (*FTO*) gene strongly associated with development of obesity (10, 18-20). Individuals homozygous for the *FTO* risk allele AA (rs9939609) weighed on average 3 kg more and had 1.7-fold increased odds of being obese compared with those homozygous for the lower-risk allele TT (21). Although there is increasing evidence that the *FTO* genetic susceptibility to obesity can be modulated by lifestyle factors such as physical activity (PA) (10, 22, 23), there is a lack of evidence on whether disclosing information on *FTO* genotype would motivate individuals to adopt more healthy lifestyles to reduce weight

(24). A recent study showed that feedback on *FTO* risk increased readiness to control weight in young and healthy adults, but no evidence of actual behavior change was found (25). The current study is part of the Food4Me intervention trial which was designed to investigate the effectiveness of different levels of personalised nutrition, including dietary, phenotypic and genotype based advice, on improving diet and health-related outcomes (14). The genotype based advice within the Food4Me trial used 5 different genetic variants each associated with a specific nutrient or phenotypic marker. However, the current study focuses on the effect of disclosing information about *FTO* genotype which was the only variant for which personalised advice for weight loss was provided. Thus, the aim of the present study was to assess the impact of disclosing personalised *FTO* based information on changes in obesity-related markers, and to investigate whether changes in obesity markers were different from those observed in other interventions groups who received non-genotype based personalised nutrition advice.

METHODS

Study design

Subjects were participants of the Food4Me Proof-of-Principle Study, a 6-month web-based RCT on personalized nutrition conducted across 7 European countries (Germany, Greece, Ireland, the Netherlands, Poland, Spain, and the UK). As outlined elsewhere (26), 1607 adults aged ≥ 18 years were included in the study. Exclusion criteria included no or limited access to the Internet, following a prescribed diet or having altered nutritional requirements because of medical conditions. Participants were screened online between August 2012 and August 2013; the characteristics of these individuals have been reported elsewhere (27).

Intervention arms

Full details of the study design have been published elsewhere (26). Briefly, participants were randomly allocated to one of 4 groups: Level 0: standard, non-personalized dietary and PA guidelines; Level 1: personalized advice based on current weight, diet and PA; Level 2: personalized advice based on current weight, diet, PA and phenotype (e.g. waist circumference [WC], blood cholesterol); and Level 3: personalized advice based on current weight, diet, PA, phenotype and genotype information for 5 genetic variants (*FTO, FADS1, TCF7L2, APOE(ε4), MTHFR*). All data were collected remotely (i.e. at home) at baseline, month 3 and month 6 following standardized operating procedures (26).

Following analysis of data collected at baseline and 3 months, participants received personalized feedback on their weight, diet and PA (Levels 1-3) or non-personalized guidelines (Level 0), depending on their randomization group, at both time points. The personalized feedback was based on pre-defined algorithms incorporating anthropometric, dietary and PA (Levels 1-3), phenotypic (Levels 2-3), and genotypic (Level 3 only) data. Results in the personalized feedback reports were indicated for each anthropometric, dietary, PA (Levels 1-3), and phenotypic (Levels 2-3) item, on 3-color graded lines (green, good; amber, improvement recommended; and red, improvement strongly recommended). In addition, all Level 3 participants received information on whether they carried the risk variant for 5 nutrition- and lifestyle-related genes (Table 1). The feedback provided for each of these five genetic variants is described in **Table 1** (26). The target nutrients or phenotypic markers related to these genotypic variants and for which participants received personalised advice were body weight for the *FTO* gene, omega 3 fatty acids intake for the *FADS1* gene, fat intake for the *TCF7L2* gene, saturated fat intake for the *APOE(ε4)* gene, and folate for the *MTHFR* gene. However, for the purposes of this study, we have included only those

participants who received genotype-based advice for the *FTO* gene, and who were advised to reduce their body weight (**Table 1, Supplemental Figure 1 and 2**).

For *FTO*, the following message was included in reports delivered to Level 3 participants:

> *"A specific variation of this gene is associated with a greater need to maintain a healthy body weight and engage in physical activity. A healthy weight combined with exercise may provide added health benefits for these individuals"*,

and Level 3 participants were informed about their *FTO* rs9939609 status i.e. whether they carried or not the risk allele ('yes' or 'no,' respectively). However, this feedback did not include any numerical information about how much extra weight an individual with a risk-conferring variant of *FTO* would be expected to carry (Supplemental Figure 1 and 2). Each personalized report (Levels 1-3) contained a specific message related to body weight, which, for Level 3 participants only, referred to *FTO*. For example, an AA/AT Level 3 participant with increased BMI and WC would read:

> *"We recommend reducing your body weight and waist circumference to a healthy normal range because you have a genetic variation that can benefit by reducing these two obesity-related markers"*.

Data collection

Participants consented to self-report their measures via the Internet and to send biological samples (buccal swabs for DNA extraction) by post, using pre-paid, stamped and addressed envelopes. To ensure that procedures were similar in all recruiting centres, standardised operating procedures were prepared for all measurements, and researchers underwent centralised training. Moreover, to enable participants to collect and report the required information and to collect, process and dispatch the biological samples correctly, participants were given detailed instructions, and video

124 demonstrations were available on the Food4Me website (www.food4me.org), in their own
125 language (26).

126 **Ethical approval and participant consent**

127 1607 participants were randomized into the study and were recruited between August 2012 and
128 August 2013 from the following centers: University College Dublin (Ireland), Maastricht
129 University (The Netherlands), University of Navarra (Spain), Harokopio University (Greece),
130 University of Reading (United Kingdom, UK), National Food and Nutrition Institute (Poland) and
131 Technical University of Munich (Germany). The Research Ethics Committees at each University
132 or Research Centre delivering the intervention granted ethical approval for the study. The
133 Food4Me trial was registered as a RCT (NCT01530139) at Clinicaltrials.gov. All participants
134 expressing an interest in the study were asked to sign online consent forms at two stages in the
135 screening process. These consent forms were automatically directed to the local study investigators
136 to be counter-signed and archived (26).

137 **Anthropometric and lifestyle measures**

138 Body weight, height and WC were self-measured and self-reported by participants via Internet.
139 Participants were instructed to measure body weight after an overnight fast, without shoes and
140 wearing light clothing using a home or commercial scale, and to measure height, barefoot, using a
141 standardised measuring tape provided by the researchers. WC was measured at the mid-point
142 between the lower rib and the iliac crest using the provided tape (26). Central obesity was defined
143 as WC >88 cm for women and >102 cm for men. BMI ($kg.m^{-2}$) was calculated from body weight
144 and height. Adiposity status was defined using World Health Organization (WHO) criteria for
145 BMI (underweight <18.5 $kg.m^{-2}$, normal weight ≥18.5 $kg.m^{-2}$ to ≤24.9 $kg.m^{-2}$, overweight ≥25.0
146 $kg.m^{-2}$ to ≤29.9 $kg.m^{-2}$ and obesity ≥30.0 $kg.m^{-2}$). Self-reported measurements were validated in a

147 sub-sample of the participants across 7 European countries and showed a high degree of reliability
148 (26).
149 Physical activity level (PAL, defined as the ratio between total energy expenditure and predicted
150 basal metabolic rate (28)) and time spent sedentary ($min.d^{-1}$) were estimated from tri-axial
151 accelerometers (TracmorD, Philips Consumer Lifestyle, The Netherlands).
152 **Genotyping**
153 Participants collected buccal cell samples at baseline using Isohelix SK-1 DNA buccal swabs and
154 Isohelix dried-capsules and posted samples to each recruiting centre for shipment to LGC
155 Genomics (Hertfordshire, United Kingdom). LGC Genomics extracted DNA and genotyped
156 specific loci using KASP^{TM} genotyping assays to provide bi-allelic scoring of *FTO* single
157 nucleotide polymorphisms (SNPs) rs9939609 and rs1121980. These two SNPs showed a high
158 linkage disequilibrium (r^2=0.96) and therefore results for rs1121980 are not reported. No
159 significant deviation from the Hardy-Weinberg Equilibrium was observed for rs9939609 (χ^2=0.51;
160 *P*=0.48).

161 **Statistical analyses**
162 In this analysis we included participants with BMI ≥25.0 $kg.m^{-2}$ and/or high WC (>88 or >102 cm,
163 for women or men, respectively) at baseline, and for whom *FTO* genotype data were available, as
164 well as anthropometrics at month 3 or month 6. These individuals were advised to reduce their
165 weight and/or WC at baseline (Levels 1-3), or would have been advised to do so (Level 0) if they
166 had not been in the control group.
167 Results from descriptive analyses are presented as means and SD for continuous variables or as
168 percentages for categorical variables. All models were adjusted for baseline outcome value, age,
169 sex and country. Multiple regression analyses were used to determine significant changes from

baseline to month 3 and baseline to month 6 for *FTO* risk (AA/AT) as well as non-risk (TT) carriers. To answer our first research question ("Does knowledge of *FTO* genotype influence changes in body weight and WC in carriers and non-carriers of the *FTO* risk allele?"), we compared Level 3 risk and non-risk carriers, for whom *FTO* genotype was disclosed, using multiple regression analysis. Our secondary research question ("Is *FTO*-based personalized advice more effective at reducing body weight and WC than non-personalized guidelines or, personalized advice based on diet or diet and phenotype alone?"), was tested using multiple regression, comparing Level 3 risk carriers (reference group) with changes observed in Level 0, Level 1, and Level 2.

Multiple imputations by fully conditional specification methods (29) were used to address missing data for body weight and WC. All statistical analyses were performed using Stata (version 14; StataCorp, College Station, TX, USA) and significance was set at $P<0.05$.

RESULTS

Study participants

A total of 5562 participants were screened online between August 2012 and August 2013; the characteristics of these individuals have been reported in the supplemental material and elsewhere (27). The first 1607 volunteers meeting the inclusion criteria were recruited to the RCT and randomized to one of the four intervention arms (**Figure 1**) (26). Only participants advised to reduce their body weight or WC at baseline (Levels 1-3), or controls who would have been advised to do so if they had not been in Level 0, were included (n=683; Figure 1). Baseline characteristics of these participants by intervention arm are shown in **Table 2**. In summary, 51% of the participants were women, mean age 43.3 (range 18 to 73 years) and mean BMI 29.3 (range 25.0

to 61.7) $kg.m^{-2}$. After 3 and 6 months, 10% and 14% of participants randomized to the intervention were lost to follow-up, respectively (Figure 1). However, intention-to-treat analyses were performed and therefore missing data for body weight and WC at month 3 and month 6 were imputed as described in the Methods section.

Changes in adiposity marker in risk and non risk carriers of the *FTO* genotype

For the overall cohort, irrespective of intervention arm (**Table 3**, analyses including all participants from L0-L3), risk carriers of the *FTO* genotype (AT/AA, n=491) achieved significantly (*P*=0.023) bigger weight reductions (-2.10 kg [95% CI: -2.49 to -1.70]) compared with non risk carriers (TT, n=192) (-1.19 kg [95% CI: -1.79 to -0.59]) at month 6. Similarly, significant differences (*P*=0.016) were observed between *FTO* genotypes for WC (-3.85 cm vs -2.46 cm for risk and non risk carriers, respectively). However, no significant differences in changes for either body weight or WC between carriers and non-carriers of the risk allele were observed at month 3 (**Supplemental Table 1**).

Effect of knowledge of *FTO* genotype on changes in obesity-related markers

These findings are restricted to participants randomized to Level 3 and who received personalised advice to reduce their body weight and/or WC. At month 3, body weight and WC were reduced significantly for both risk and non-risk carriers of the *FTO* gene in Level 3 (**Supplemental Table 2**). However, there were no significant effects of disclosure of *FTO* risk on changes in obesity-related markers at month 3 (**Supplemental Table 2** and **Supplemental Figure 3**). Furthermore, in Level 3, nearly twice as many participants carrying the risk allele lost at least 5% body weight as non-risk carriers (14.2 and 7.6 %, respectively) (**Supplemental Table 2**).

Similarly, body weight and WC were significantly reduced from baseline to month 6 in both risk and non-risk carriers of the *FTO* risk allele, who were randomized to Level 3 (**Table 3**). Moreover, significant differences were found between Level 3 risk and non-risk carriers of the *FTO* gene for each of the obesity-related outcomes; reductions in body weight and WC were almost twice as large in Level 3 risk carriers (-2.28 kg and -4.34 cm) compared with Level 3 non-risk carriers (-1.19 kg and -1.99 cm) (**Table 3**). Furthermore, 16.2% of Level 3 non-risk carriers compared with 27.4% of the risk carriers, achieved a weight loss >5% at month 6. Similar results were observed for WC (**Table 4**). Although there was no significant interaction between *FTO* genotype and intervention arm for body weight (P=0.641) or WC (P=0.523), larger reductions in obesity-related traits were observed for *FTO* risk carriers, compared with non-risk carriers, in Levels 0-2 where participants had no knowledge of their genotype (**Figure 2**).

Effect of *FTO*-based personalized advice on obesity-related markers compared with other forms of personalization

Significant reductions in WC were observed at month 3 in Levels 0 (-1.67 cm), 1 (-2.10 cm) and 2 (-2.14 cm) participants, who were not stratified by *FTO* genotype. However, these changes were lower than those observed for Level 3 risk carriers (-3.47 cm). The WC reduction in Level 3 risk carriers was significantly greater than for participants in Level 0 (P=0.015), Level 1 (P=0.039) and for Level 2 (P=0.046) who were not stratified by their *FTO* genotype. However, none of these findings remained significant after correction for multiple testing (using P<0.01). Participants in Levels 0, 1 and 2 also showed significant reductions in weight (**Supplemental Table 3**). At month 6, there were significant reductions in body weight and WC for participants in all intervention groups (**Table 5**).

DISCUSSION

Main findings

The main findings of this study were: a) both non-personalized and personalized forms of advice were effective at reducing body weight and WC after a 6-month intervention and b) compared with the control group, those in Level 3 who were *FTO* risk carriers had significantly greater reductions in body weight (-1.34 vs -2.28 kg, p=0.045) and WC (-2.82 vs -4.34 cm, p=0.046). However, the magnitude of changes observed in Level 1 and 2, who received non-genetic based personalized advice, for body weight (-2.08 and -1.96 kg, respectively) and WC (-3.51 and -3.63 cm, respectively) was similar to those observed in Level 3 FTO risk carriers (p>0.05).

Comparison with other studies

In the last decade, there has been growing interest in tailoring lifestyle interventions using personal DNA information (30). It has been hypothesized that providing lifestyle advice based on genetic information would motivate people to make behavioral changes favorable for disease prevention, beyond what could be achieved with non-gene-based tailored programs. In a recent meta-analysis, Hollands *et al.*,(31) reported no effect of adding DNA-based disease risk estimates compared with a non-DNA based approach for interventions aiming at smoking cessation (six studies; n=2663), improving diet (seven studies; n=1784), and increasing physical activity (six studies; n=1704). The authors concluded that evidence supporting gene-based interventions for behavior change is lacking. Existing data come from studies with predominantly high or unclear risk of bias, and where the evidence was typically of low quality. Therefore, larger and better quality studies should be performed to elucidate the effect of personalized advice based on genetic information (31).

The evidence in favor of gene-based lifestyle advice is limited. Arkadianos *et al.* reported that participants in a traditional weight management diet group and participants receiving a nutrigenetically tailored diet both lost similar amounts of weight at 100-300 days of follow up. Thereafter, participants in the nutrigenetic group were significantly more likely to maintain their weight loss compared with the control group (32). In contrast, there were no short-term (~3 months) or longer-term (~1 year) changes in self-reported anxiety, or exercise, in generally healthy adults receiving information from a commercial direct-to-consumer genome-wide risk test (33, 34). However, this study reported changes in fat intake for those individuals who received increased obesity risk feedback (33). Frankwich and colleagues observed no between-group differences in weight loss in a small study of American veterans randomly assigned either to a genetics-guided therapy group, where participants received one of four diets (balanced, low-carbohydrate, low-fat or Mediterranean) based on their risk status for seven obesity-related SNPs (*APOA2*, *ADIPOQ*, *FTO*, *KCTD10*, *LIPC*, *MMAB* and *PPARG*), or to a standard therapy group, where participants followed a balanced diet (35). Furthermore, Meisel *et al*. showed that healthy individuals receiving feedback on *FTO* status in their weight control advice felt more prepared to control their weight but this had no greater effect on behavior than weight control advice alone (25). Our results are in line with studies outlined above. We observed that the magnitude of weight and WC reductions was similar in all three groups receiving personalized advice; adding gene-based advice did not seem to promote adiposity changes beyond what was achieved by tailored feedback based on diet or diet and phenotype alone.

Although differences in weight and WC reductions were almost twice as large in individuals informed of their risk for *FTO*, compared with those informed of their absence of *FTO*-related risk, there was no clear evidence that risk knowledge played a role. Surprisingly, *FTO* risk carriers,

irrespective of their intervention group, had greater improvements in obesity-related markers than non-risk carriers. This was an unexpected and rather counter-intuitive finding. All other factors being equal (same environment), one would expect that individuals who are genetically (and/or epigenetically) predisposed to obesity would have to make greater efforts to counter this predisposition and to achieve similar weight loss as other obese individuals who are not genetically predisposed. Alternatively, the fact that carriers of the *FTO* risk allele were slightly heavier than non-risk carriers may mean that they have greater motivation to lose weight when compared with participants with no copies of the *FTO* risk variant, who were lighter at baseline. For example, in a relatively small study of 51 obese or overweight U.S. veterans, Frankwich and colleagues observed that participants who had low-risk polymorphisms for obesity lost more weight than all other participants at 8 weeks and had significantly greater reductions in BMI and WC at 24 weeks (35). However, these finding are in disagreement with a recent meta-analysis conducted using 9563 individual participant data from eight randomized controlled trials. This study found that the *FTO* genotype had no detectable effect on weight loss in overweight and obese adults in response to lifestyle or drug-based intervention (36).

Strengths and limitations

The Food4Me study is the largest Internet-based intervention on personalized nutrition to date. Innovative aspects of the Food4Me Study include the creation of algorithms for delivering tailored lifestyle advice based on participant characteristics including behavioral, phenotypic and genotypic information. Another strength of the study was the delivery of the intervention across 7 European countries via the Internet and application of a remote system for data and biological sample collection. Our Internet-based platform was effective in retaining participants; 85%

completed month 6 follow up and there was > 98% compliance with DNA testing, which is high compared with previous web-based survey research (37) and web-based (34) or face-to-face (25) genetic-based interventions. In a study of direct-to-consumer genomic testing, Bloss *et al*. reported 44% and 63% dropouts at months 3 and 12, respectively (33, 34). Moreover, the profile of those interested in participating in the Food4Me intervention study was similar to that of European adults (26), most of whom would benefit from improved diet and more PA. Finally, we used multiple imputation procedures to address missing data and so maximized the amount of useful information available from the 683 participants in the part of the Food4Me Study.

Our limitations include that we did not investigate how participants perceived the DNA-based feedback. Given that Food4Me was an intervention targeting multiple, dietary and lifestyle behaviors, the impact of the genotypic results might have been diluted by the volume of other information provided. Moreover, the genetic feedback was "only" a positive reinforcement, i.e. that participants with the higher-risk genotype would benefit more by reducing their weight and WC. The greater risk for obesity and associated co-morbidities was not stressed in the reports and it is possible that the impact of such feedback would have been stronger. Additionally, some of the analyses performed by intervention arm and *FTO* genotype in this investigation of secondary outcomes may not have the statistical power to detect biologically/ clinically-relevant differences in adiposity. Larger studies are needed to corroborate these findings. Finally, height, weight, and WC were self-reported but a concurrent validation study showed that the self-reported anthropometric measures were reliable (38).

Conclusion

Larger reductions in body weight and WC were observed for risk carriers compared with non-risk carriers of the *FTO* gene. However, changes in these obesity-related traits were similar in all

groups receiving personalized advice. Adding genetic information to the tailored feedback did not enhance the effectiveness of the intervention, compared with personalization based on diet or diet and phenotype alone. Our personalized Internet-based intervention was effective at recruiting and retaining participants. This offers promise as a scalable and sustainable route to improve behaviors with important public health benefits (11).

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REFERENCES

1. OECD. Health at a Glance: Europe 2012. 2012. Internet: http://dx.doi.org/10.1787/9789264183896-en accessed Date Accessed)|.
2. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet 2014.
3. WHO. Global Helath Risk: mortality and burden of disease attributable to selected major risk World Health Organization, 2009.
4. Ezzati M, Riboli E. GLOBAL HEALTH Behavioral and Dietary Risk Factors for Noncommunicable Diseases. New England Journal of Medicine 2013;369(10):954-64. doi: 10.1056/NEJMra1203528.
5. Ekelund U, Ward HA, Norat T, Luan J, May AM, Weiderpass E, Sharp SJ, Overvad K, Ostergaard JN, Tjonneland A, et al. Physical activity and all-cause mortality across levels of overall and abdominal adiposity in European men and women: the European Prospective Investigation into Cancer and Nutrition Study (EPIC). The American journal of clinical nutrition 2015;101(3):613-21. doi: 10.3945/ajcn.114.100065.
6. Hurling R, Catt M, Boni MD, Fairley BW, Hurst T, Murray P, Richardson A, Sodhi JS. Using internet and mobile phone technology to deliver an automated physical activity program: randomized controlled trial. Journal of medical Internet research 2007;9(2):e7. doi: 10.2196/jmir.9.2.e7.
7. Alexander GL, McClure JB, Calvi JH, Divine GW, Stopponi MA, Rolnick SJ, Heimendinger J, Tolsma DD, Resnicow K, Campbell MK, et al. A randomized clinical trial evaluating online interventions to improve fruit and vegetable consumption. American journal of public health 2010;100(2):319-26. doi: 10.2105/AJPH.2008.154468.
8. Hansen AW, Gronbaek M, Helge JW, Severin M, Curtis T, Tolstrup JS. Effect of a Web-based intervention to promote physical activity and improve health among physically inactive adults: a population-based randomized controlled trial. Journal of medical Internet research 2012;14(5):e145. doi: 10.2196/jmir.2109.
9. Livingstone KM, Celis-Morales C, Navas-Carretero S, San-Cristobal R, Macready AL, Fallaize R, Forster H, Woolhead C, O'Donovan CB, Marsaux CFM, et al. Effect of an Internet-based, personalized nutrition randomized trial on dietary changes associated with the Mediterranean diet: the Food4Me Study. American Journal of Clinical Nutrition 2016;104(2):288-97. doi: 10.3945/ajcn.115.129049.
10. Celis-Morales C, Marsaux CFM, Livingstone KM, Navas-Carretero S, San-Cristobal R, O'Donovan CB, Forster H, Woolhead C, Fallaize R, Macready AL, et al. Physical activity attenuates the effect of the FTO genotype on obesity traits in European adults: The Food4Me study. Obesity 2016;24(4):962-9. doi: 10.1002/oby.21422.
11. Celis-Morales C, Lara J, Mathers JC. Personalising nutritional guidance for more effective behaviour change. The Proceedings of the Nutrition Society 2015;74(2):130-8. doi: 10.1017/S0029665114001633.

12. Hutchesson MJ, Rollo ME, Krukowski R, Ells L, Harvey J, Morgan PJ, Callister R, Plotnikoff R, Collins CE. eHealth interventions for the prevention and treatment of overweight and obesity in adults: a systematic review with meta-analysis. Obesity reviews : an official journal of the International Association for the Study of Obesity 2015;16(5):376-92. doi: 10.1111/obr.12268.
13. Levine DM, Savarimuthu S, Squires A, Nicholson J, Jay M. Technology-assisted weight loss interventions in primary care: a systematic review. Journal of general internal medicine 2015;30(1):107-17. doi: 10.1007/s11606-014-2987-6.
14. Celis-Morales C, Livingstone KM, Marsaux CFM, Macready AL, Fallaize R, O'Donovan CB, Woolhead C, Forster H, Walsh MC, Navas-Carretero S, et al. Effect of personalized nutrition on health-related behaviour change: evidence from the Food4me European randomized controlled trial. International Journal of Epidemiology 2016. doi: 10.1093/ije/dyw186.
15. Collins FS, Varmus H. A New Initiative on Precision Medicine. New England Journal of Medicine 2015;30:3. doi: 10.1056/NEJMp1500523.
16. Marteau TM, Lerman C. Genetic risk and behavioural change. Bmj 2001;322(7293):1056-9.
17. Marteau TM, French DP, Griffin SJ, Prevost AT, Sutton S, Watkinson C, Attwood S, Hollands GJ. Effects of communicating DNA-based disease risk estimates on risk-reducing behaviours. Cochrane Database of Systematic Reviews 2010(10). doi: 10.1002/14651858.CD007275.pub2.
18. Loos RJ, Bouchard C. FTO: the first gene contributing to common forms of human obesity. Obesity reviews : an official journal of the International Association for the Study of Obesity 2008;9(3):246-50. doi: 10.1111/j.1467-789X.2008.00481.x.
19. Loos RJ, Yeo GS. The bigger picture of FTO: the first GWAS-identified obesity gene. Nature reviews Endocrinology 2014;10(1):51-61. doi: 10.1038/nrendo.2013.227.
20. Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, Powell C, Vedantam S, Buchkovich ML, Yang J, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature 2015;518(7538):197-206. doi: 10.1038/nature14177.
21. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, et al. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 2007;316(5826):889-94. doi: 10.1126/science.1141634.
22. Kilpelaeinen TO, Qi L, Brage S, Sharp SJ, Sonestedt E, Demerath E, Ahmad T, Mora S, Kaakinen M, Sandholt CH, et al. Physical Activity Attenuates the Influence of FTO Variants on Obesity Risk: A Meta-Analysis of 218,166 Adults and 19,268 Children. Plos Medicine 2011;8(11). doi: 10.1371/journal.pmed.1001116.
23. Young AI, Wauthier F, Donnelly P. Multiple novel gene-by-environment interactions modify the effect of FTO variants on body mass index. Nature Communications 2016;7:12724. doi: 10.1038/ncomms12724.
24. Marsaux CFM, Celis-Morales C, Livingstone KM, Fallaize R, Kolossa S, Hallmann J, San-Cristobal R, Navas-Carretero S, O'Donovan CB, Woolhead C, et al. Changes in Physical Activity Following a Genetic-Based Internet-Delivered

Personalized Intervention: Randomized Controlled Trial (Food4Me). Journal of Medical Internet Research 2016;18(2):e30. doi: 10.2196/jmir.5198.
25. Meisel SF, Beeken RJ, van Jaarsveld CH, Wardle J. Genetic susceptibility testing and readiness to control weight: Results from a randomized controlled trial. Obesity 2015;23(2):305-12. doi: 10.1002/oby.20958.
26. Celis-Morales C, Livingstone KM, Marsaux CF, Forster H, O'Donovan CB, Woolhead C, Macready AL, Fallaize R, Navas-Carretero S, San-Cristobal R, et al. Design and baseline characteristics of the Food4Me study: a web-based randomised controlled trial of personalised nutrition in seven European countries. Genes & nutrition 2015;10(1):450. doi: 10.1007/s12263-014-0450-2.
27. Livingstone K, Celis-Morales C, Navas-Carretero S, San-Cristobal R, O'Donovan C, Forster H, Woolhead C, Marsaux CM, Macready A, Fallaize R, et al. Profile of European adults interested in internet-based personalised nutrition: the Food4Me study. European Journal of Nutrition 2015:1-11. doi: 10.1007/s00394-015-0897-y.
28. Henry CJ. Basal metabolic rate studies in humans: measurement and development of new equations. Public health nutrition 2005;8(7A):1133-52.
29. Liu Y, De A. Multiple Imputation by Fully Conditional Specification for Dealing with Missing Data in a Large Epidemiologic Study. International journal of statistics in medical research 2015;4(3):287-95. doi: 10.6000/1929-6029.2015.04.03.7.
30. Nielsen DE, El-Sohemy A. Applying genomics to nutrition and lifestyle modification. Personalized Medicine 2012;9(7):739-49. doi: 10.2217/pme.12.79.
31. Hollands GJ, French DP, Griffin SJ, Prevost AT, Sutton S, King S, Marteau TM. The impact of communicating genetic risks of disease on risk-reducing health behaviour: systematic review with meta-analysis. Bmj-British Medical Journal 2016;352. doi: 10.1136/bmj.i1102.
32. Arkadianos I, Valdes AM, Marinos E, Florou A, Gill RD, Grimaldi KA. Improved weight management using genetic information to personalize a calorie controlled diet. Nutrition journal 2007;6:29. doi: 10.1186/1475-2891-6-29.
33. Bloss CS, Schork NJ, Topol EJ. Effect of Direct-to-Consumer Genomewide Profiling to Assess Disease Risk. New England Journal of Medicine 2011;364(6):524-34. doi: 10.1056/NEJMoa1011893.
34. Bloss CS, Wineinger NE, Darst BF, Schork NJ, Topol EJ. Impact of direct-to-consumer genomic testing at long term follow-up. Journal of Medical Genetics 2013;50(6):393-400. doi: 10.1136/jmedgenet-2012-101207.
35. Frankwich KA, Egnatios J, Kenyon ML, Rutledge TR, Liao PS, Gupta S, Herbst KL, Zarrinpar A. Differences in Weight Loss Between Persons on Standard Balanced vs Nutrigenetic Diets in a Randomized Controlled Trial. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association 2015;13(9):1625-32 e1; quiz e145-6. doi: 10.1016/j.cgh.2015.02.044.
36. Livingstone KM, Celis-Morales C, Papandonatos GD, Erar B, Florez JC, Jablonski KA, Razquin C, Marti A, Heianza Y, Huang T, et al. FTO genotype and weight loss: systematic review and meta-analysis of 9563 individual participant data from eight randomised controlled trials. Bmj-British Medical Journal 2016;354. doi: 10.1136/bmj.i4707.

37. Yetter G, Capaccioli K. Differences in responses to Web and paper surveys among school professionals. Behavior Research Methods 2010;42(1):266-72. doi: 10.3758/brm.42.1.266.
38. Celis-Morales C, Livingstone KM, Woolhead C, Forster H, O'Donovan CB, Macready AL, Fallaize R, Marsaux CFM, Tsirigoti L, Efstathopoulou E, et al. How reliable is internet-based self-reported identity, socio-demographic and obesity measures in European adults? Genes & nutrition 2015;10(5):476-. doi: 10.1007/s12263-015-0476-0.

Table 1. Genetic feedback delivered to participants randomized to Level 3[1]

Genes	Targeted recommendation	Nutritional influences associated with some variations of this gene	Do you have the genetic variation that can be modified by dietary change?
FTO	Reduce body weight	A specific variation of this gene is associated with a greater need to maintain a healthy body weight and engage in physical activity. A healthy weight combined with exercise may provide added health benefits for these individuals.	Yes / No
FADS1	Increase Omega 3 intake	People with a specific variation of this gene can benefit by increasing their intake of the healthy omega-3 fat found in oily fish. Increasing omega-3 intake has been associated with an improvement in factors relating to cardiovascular health in these individuals.	Yes / No
TCF7L2	Reduce fat intake	A specific variation of this gene is associated with improved weight loss when following a low fat diet compared to other weight loss diets. Reducing dietary fat may enhance weight loss in these individuals.	Yes / No
ApoE(e4)	Reduce saturated fat intake	A specific variation of this gene is associated with a greater need to maintain healthy cholesterol levels. Decreasing saturated fat intake has been associated with an improvement in cholesterol and factors relating to cardiovascular health in these individuals.	Yes / No
MTHFR	Increase folate intake	People with a specific variation of this gene can benefit by increasing their intake of the vitamin folate. Increasing folate intake (found in green leafy vegetables) has been associated with an improvement in factors relating to cardiovascular health in these individuals.	Yes / No

[1]Genetic information provided to participants randomized to the "Level 3" and who received personalised advice based on diet, phenotypic markers and these genetic markers.

Table 2. Baseline characteristics of the Food4Me participants with high BMI or WC by intervention arm[1]

	Level 1 "Control"	**Level 1** "Diet"	**Level 2** "Diet + Phenotype"	**Level 3** *FTO* Non-risk (TT)	**Level 3** *FTO* Risk (AT/AA)
Total (n) BMI ≥25.0 $kg.m^{-2}$	171	153	173	47	139
Total (n) WC >88 or >102 cm for women and men respectively	84	82	96	27	71
Sex - women (%)	53.8	49.0	47.4	48.9	54.6
Age (years)	42.9 (12.2)	44.2 (11.4)	43.9 (12.1)	42.2 (13.3)	43.7 (11.9)
Anthropometrics					
Weight (kg)	85.1 (12.6)	87.5 (15.0)	87.3 (12.8)	83.9 (12.4)	86.1 (12.9)
BMI ($kg.m^{-2}$)	29.0 (3.8)	29.7 (4.5)	29.8 (3.9)	28.7 (3.1)	29.4 (4.3)
WC (cm)	95.7 (11.1)	96.0 (0.12)	96.9 (11.6)	94.2 (10.8)	96.1 (11.0)
Physical Activity					
PAL[2]	1.69 (0.13)	1.72 (0.16)	1.70 (0.16)	1.71 (0.13)	1.69 (0.13)
Sedentary time ($min.day^{-1}$)	761.9 (77.5)	761.9 (73.9)	761.0 (84.2)	756.5 (74.7)	767.7 (79.4)

[1]Level 0 received non-personalized advice. Levels 1, 2, and 3 received personalized advice based on Diet, Diet + Phenotype, or Diet + Phenotype + Genotype, respectively. Baseline characteristics for all interventions arms include only participants with a BMI ≥25.0 and/or WC >88 cm and >102 cm for women and men, respectively.

[2]PAL, physical activity level (ratio between total energy expenditure and basal metabolic rate);

Table 3. Changes in obesity-related markers at month 6 in risk and non-risk carriers of the FTO genotype[1]

	FTO **non-risk (TT)**	***FTO*** **risk (AT/AA)**	*P*-value for difference in change between risk and non-risk carriers
Analysis including participants from L0-L3[2]			
Weight (kg)			
n	192	491	
Delta	-1.19*	-2.10*	*P*=0.023
(95% CI)	(-1.79, -0.59)	(-2.49; -1.70)	
WC (cm)			
n	107	252	
Delta	-2.46*	-3.85 *	*P*=0.016
(95% CI)	(-3.40, -1.51)	(-4.49; -3.21)	
Analysis restricted to participants in L3[3]			
Weight (kg)			
n	47	139	
Delta	-1.19*	-2.28*	*P*=0.037
(95% CI)	(-2.19, -0.19)	(-3.06, -1.48)	
WC (cm)			
n	27	71	
Delta	-1.99*	-4.34*	*P*=0.048
(95% CI)	(-4.04, -0.05)	(-5.63, -3.08)	

[1]Data presented as delta and 95% confidence interval. Significant changes between baseline and month 6: *$P<0.01$; Models were adjusted for country, age, sex, and baseline outcome measures. Intervention arm was included as an additional covariate in the analysis. Deltas were calculated as [month 6 – baseline]. [2]These analyses pooled participants from all interventions groups (control, L1, L2 and L3) who were advised to loss body weight or to reduce their WC, irrespective of whether they were informed or not of their genetic risk. [3]These analyses were restricted to those participants randomized to Level 3 and who were informed of their *FTO* genotype (risk or non risk) and who were advised to loss body weight or reduce their WC. Significant changes in the outcomes from baseline were tested using multiple regression analysis. Differences in the outcomes delta between risk and non-risk carriers were tested using regression analysis.

Table 4. Percentage of participants who achieved 2.5%, 5% and 10% weight loss or waist circumference (WC) reduction by intervention arm at month 6[1]

	Level 1 "Control"	**Level 1** "Diet"	**Level 2** "Diet + Phenotype"	**Level 3** *FTO* non risk (TT)	**Level 3** *FTO* risk (AT/AA)
Weight (kg)					
n	171	153	173	47	139
2.5% to 4.9%	20.5	20.4	15.4	21.6	21.7
5.0% to 9.9%	13.0	11.8	18.8	16.2	21.8
≥10%	4.8	8.7	4.0	0	5.6
WC (cm)					
n	84	82	96	27	71
2.5% to 4.9%	20.0	16.7	24.2	13.5	16.3
5.0% to 9.9%	14.5	16.7	24.2	13.5	22.6
≥10%	6.2	9.5	6.8	2.7	8.1

[1]Data presented as percentages. No formal comparisons between groups were made for results presented in this table. These analyses were restricted to participants who were advised to lose body weight or to reduce their WC.

Table 5. Changes in obesity-related markers at month 6 in Level 3 (*FTO* risk and non risk carriers) compared with participants in Levels 0, 1 or 2 who did not receive genotype advice[1]

	Intervention arms					*P-value*		
	Level 0 "Control group"	**Level 1** "Diet"	**Level 2** "Diet + Phenotype"	**Level 3** *FTO* non risk (TT)	**Level 3** *FTO* risk (AT/AA)	L3 *FTO* risk vs Control	L3 *FTO* risk vs L1	L3 *FTO* risk vs L2
Weight (kg)								
n	171	153	173	47	139			
Delta	-1.34	-2.08	-1.96	-1.19	-2.28	0.045	0.752	0.602
(95% CI)	(-2.02, -0.66)*	(-2.83, -1.31)*	(-2.54, -1.37)*	(-2.19, -0.19)*	(-3.06, -1.48)*			
WC (cm)								
n	84	82	96	27	71			
Delta	-2.82	-3.51	-3.63	-1.99	-4.34	0.046	0.290	0.361
(95% CI)	(-3.86, -1.78)*	(-4.82, -2.21)*	(-4.54, -2.72)*	(-4.04, -0.05)*	(-5.63, -3.08)*			

[1]Data presented as delta and 95% confidence interval. Significant changes between baseline and month 6: *$P<0.001$. The p-value was corrected for multiple testing and significant differences were set as $p<0.01$. Models were adjusted for country, age, sex, and baseline outcome measures. Deltas were calculated as [month 6 – baseline]. Significant changes in the outcomes from baseline and differences within the each level were tested using multiple regression analysis. These analyses were restricted to participants in Levels 0, 1 and 2 who were advised to lose body weight and/or to reduce their WC and who were not stratified by *FTO* genotype for comparison with participants in Level 3 who were further stratified as *FTO* risk and non risk carriers.

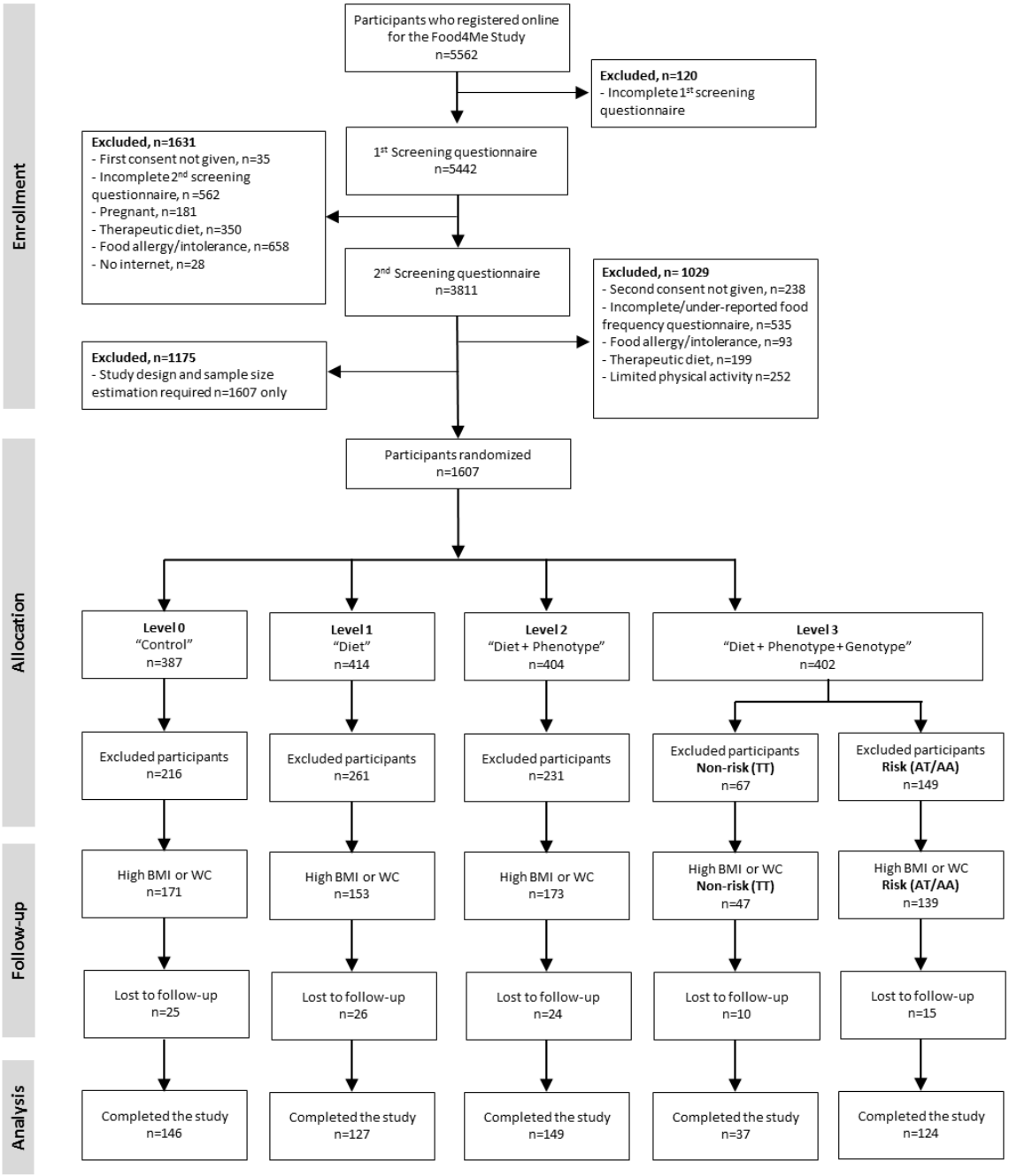

Figure 1. CONSORT diagram

BMI, body mass index; WC, waist circumference. Participants in Level 0 received non-personalized advice, whereas participants in Levels 1-3 received personalized advice, during the intervention. Participants in Levels 1-3 with high BMI (≥25 $kg.m^{-2}$) or WC (>88 or 102 cm for women or men, respectively) at baseline were advised to reduce their body weight. For analyses, Level 3 was stratified based on *FTO* genotype (TT: non-risk and AA, AT: risk).

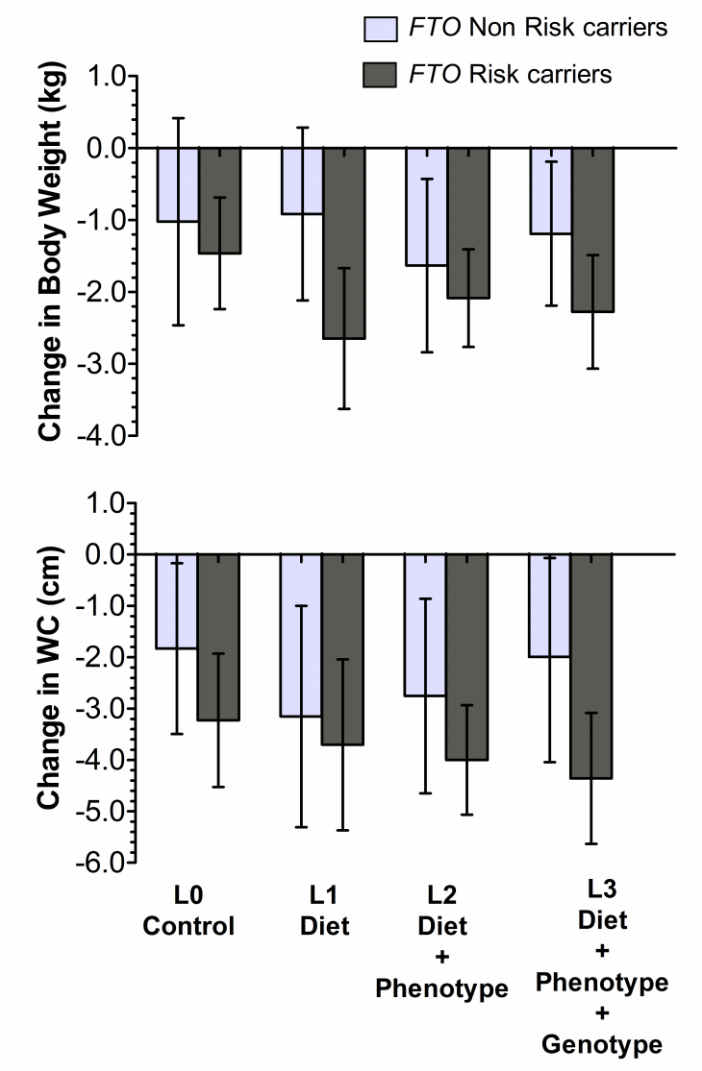

Figure 2. Changes in obesity-related markers at month 6 by intervention arm and *FTO* genotype

Data are presented as mean delta from baseline and 95% confidence interval. Non risk and risk carriers across all intervention Levels reduced their waist circumference at month 6 compared with month 0 (P<0.001). Similar reductions were observed for body weight except for non risk carriers in Levels 0 and 1. No significant interactions were observed between intervention arm and *FTO* genotype for any of the outcomes. Analyses were adjusted for age, sex, country and outcome values at baseline. WC, waist circumference. The interaction between intervention arm and *FTO* genotype was tested using regression analysis (*P*=0.641 and *P*=0.523 for body weight and WC, respectively). Participants included in the analysis were restricted to those advised to reduce their body weight and/or WC. Numbers of participants included for body weight non risk and risk carriers, respectively,

were L0 n=46’ 124; L1 n=50, 101; L2 n=48, 125; L3 n=47, 139. Numbers for WC non risk and risk carriers, respectively, were L0 n=24, 59; L1 n=28, 54, L2 n=28, 68; L3 n=27, 71.