Influence of SNPs in nutrient-sensitive candidate genes and gene–diet interactions on blood lipids: the DiOGenes study

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Abstract

Blood lipid response to a given dietary intervention could be determined by the effect of diet, gene variants or gene–diet interactions. The objective of the present study was to investigate whether variants in presumed nutrient-sensitive genes involved in lipid metabolism modified lipid profile after weight loss and in response to a given diet, among overweight European adults participating in the Diet Obesity and Genes study. By multiple linear regressions, 240 SNPs in twenty-four candidate genes were investigated for SNP main and SNP–diet interaction effects on total cholesterol, LDL-cholesterol, HDL-cholesterol and TAG after an 8-week low-energy diet (only main effect), and a 6-month *ad libitum* weight maintenance diet, with different contents of dietary protein or glycaemic index. After adjusting for multiple testing, a SNP–dietary protein interaction effect on TAG was identified for lipin 1 (*LPIN1*) rs4315495, with a decrease in TAG of −0·26 mmol/l per A-allele/protein unit (95 % CI −0·38, −0·14, P=0·000043). In conclusion, we investigated SNP–diet interactions for blood lipid profiles for 240 SNPs in twenty-four candidate genes, selected for their involvement in lipid metabolism pathways, and identified one significant interaction between *LPIN1* rs4315495 and dietary protein for TAG concentration.

Key words: Blood lipids: Gene–diet interactions: Protein: Glycaemic index: SNPs

Dyslipidaemia is an important risk factor for CVD[1]. Overweight and obesity, in particular intra-abdominal fat deposition, is associated with decreased HDL-cholesterol (HDL-C) and hypertriacylglycerolaemia[1]. Plasma concentrations of total cholesterol (TC), LDL-cholesterol (LDL-C), HDL-C and TAG are modified by diet, physical activity and smoking status[1]. However, there are considerable inter-individual differences in metabolic susceptibility to these lifestyle factors[2,3]. These differences may partly be determined by genetic factors, and several genome-wide association studies (GWAS) have identified loci that are associated with blood lipid concentrations[4–15]. Still, these loci only explain a part of the variance in lipid profiles, and this could be partly due to gene–gene and gene–environment interaction effects[16], as previous candidate gene studies have suggested interaction effects between genes and lifestyle for dyslipidaemia[17,18].

The objective of the present study was to investigate whether SNPs in nutrient-sensitive genes involved in lipid metabolism modified lipid profile after weight loss and in response to a given diet. We analysed 240 SNPs in twenty-four presumed nutrient-sensitive candidate genes among overweight European adults participating in the Diet Obesity and Genes (DiOGenes) study. SNP main effects on TC, LDL-C, HDL-C and TAG were examined after an 8-week low-energy diet (LED), and both SNP main and SNP–diet interaction effects were examined after a 6-month *ad libitum* weight maintenance diet, with different contents of dietary protein or glycaemic index (GI).

Materials and methods

The DiOGenes study (www.DiOGenes-eu.org) is a Pan-European randomised dietary intervention study exploring the effect of diets with different contents of protein and GI on weight regain and metabolic health after weight loss. The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the local ethical committee in the respective country. Written informed consent was obtained from all subjects. The participants were overweight or obese (BMI 27–45 kg/m²) but otherwise healthy, with no heart disease, diabetes, severe dyslipidaemia or hypertension. The present trial has been registered at ClinicalTrials.gov (identification no. NCT00390637).

Design and methods have been described in detail previously[19]. In brief, participants who had lost ≥8% of their initial body weight after an 8-week LED (3400–4200 kJ/d) were randomised to one of five different 6-month *ad libitum* weight maintenance diets based on either combinations of low/high dietary protein (LP/HP) and low/high GI (LGI/HGI), or a control diet according to national dietary guidelines: (1) LP/LGI; (2) LP/HGI; (3) HP/LGI; (4) HP/HGI; (5) control diet. The target for the dietary intervention was a difference of 12% of total energy consumed from protein between the HP and LP diets and a difference of 15 GI units between the HGI and LGI diets. The actual differences between the respective diets calculated from diet registrations were 5·4% of energy consumed from protein and 5 GI units[20].

TC, HDL-C and TAG were analysed at the Department of Clinical Biochemistry, Gentofte University Hospital, Denmark, and LDL-C was calculated using Friedewald's equation[21].

Initially, sixty-nine candidate nutrient-sensitive genes were selected based on prior knowledge of whether the pathway, gene, gene transcript or SNP was implicated in obesity, weight loss, weight regain or associated diseases with emphasis on the interaction with dietary protein or GI, from literature search and the TUB database at IntegraGen (Evry, France) for the purpose of investigating their role in body weight regulation during dietary treatment. For the presumed nutrient-sensitive candidate genes, a comprehensive approach was used to ensure genetic coverage of the locus (± 5 kb) by selecting tagSNPs for each of the selected genes. TagSNPs were identified from the International HapMap data for European ancestry (release 20, NCBI build 35), and linkage disequilibrium (LD) structure was evaluated using Haploview software, version 3.32 (Broad Institute of MIT and Harvard, MA, USA)[22]. TagSNPs were selected using Tagger[23] with a single marker option with an LD threshold of r^2 0·7–0·8. SNPs located in exonic regions, frequently studied, or included in the Illumina HumanHap 300 were preferentially included as tagSNPs, while SNPs with an expected low genotyping success rate (in close proximity to another SNP (60 bp) or in a repeat region) were deselected. In total, 768 tagSNPs were selected for genotyping (Table S1, available online). Among the selected nutrient-sensitive genes, the genes that were known from the literature to be involved in lipid metabolism were included in the analyses; these twenty-four genes included: adiponectin (*ADIPOQ*)[24]; β2/β3-adrenergic receptor (*ADRB2,3*)[25]; activating transcription factor 6 (*ATF6*)[26]; basic helix-loop-helix family, member e40 (*BHLHE40*)[27]; caveolin 1 (*CAV1*)[28]; CCAAT/enhancer binding protein (*CEBPB*)[29]; cathepsin S (*CTSS*)[30]; fatty acid-binding protein 1 (*FABP1*)[31,32]; fatty acid-binding protein 4 (*FABP4*)[33]; farnesyltransferase (*FNTA*)[34]; leptin (*LEP*)[35]; lipin 1, 2, 3 (*LPIN1, 2, 3*)[36,37]; lipoprotein lipase (*LPL*)[38]; Max-like protein (MLX) interacting protein-like (*MLXIPL*)[39]; matrix metallopeptidase 9 (*MMP9*)[40]; microsomal TAG transfer protein (*MTTP*)[41]; nuclear receptor subfamily 1, group I, member 2 (*NR1I2*)[42]; phosphoenolpyruvate carboxykinase 2 (*PCK2*)[43]; PPARγ co-activator 1-α (*PPARGC1A*)[44]; PPARδ (*PPARD*)[45]; PPARγ (*PPARG*)[46].

Genotyping of all samples was performed using the Illumina® Bead Station System (Illumina, Inc.) by IntegraGen with CEPH (Human Polymorphism Study Center) controls (reproducibility: 100%; concordance rate: 99·6%). A total of 240 tagSNPs with a call rate ≥95%, a minor allele frequency (MAF) >1% and without significant (*P* > 0·001) deviations from Hardy–Weinberg equilibrium were included in the main analyses. Genotype analyses were performed and reported with respect to the minor allele, here defined as the risk allele.

Statistical analyses

By multiple linear regression analyses, we examined the following: (1) SNP main effects on changes in blood lipids after an 8-week LED; (2) SNP main effects on changes in

blood lipids after the 6-month *ad libitum* weight maintenance diet; (3) SNP–diet interaction effects on changes in blood lipids for HP *v.* LP and HGI *v.* LGI after the 6-month *ad libitum* weight maintenance diet. SNP main effects on TC, HDL-C, LDL-C and TAG at baseline were also examined but merely at an explorative level, as the present study was not initially designed to investigate baseline associations.

Before the multiple linear regression analyses, the five-level diet variables were recoded into indicator variables for levels of protein intake and GI (and for the control diet); additive genetic models were assumed and corresponding SNP main-effect, diet main-effect and SNP–diet (product-based) interaction variables were created and used for the analyses. Models were adjusted for baseline age, BMI and waist circumference, sex, smoking status, partner, weight loss (after the LED) and weight regain (after the *ad libitum* diet). Furthermore, we adjusted for period length of the LED and the *ad libitum* diet, where some variation from the intended duration occurred. The LGI or LP served as reference groups of main interest and control diet status was included in models but not of main focus, due to the variation in diets between countries.

Based on the available sample sizes (Table 1), we performed power calculations in the form of least detectable effects based on the assumption of significance levels and powers of 5 and 80%, respectively. The analysis of SNP main effects associated with gains during the LED phase led to least detectable effects of 0·34 (MAF = 0·05) and 0·15 (MAF = 0·45) in units of standard deviations of the outcome. Similarly, the case of SNP main-effects analysis related to gains during the *ad libitum* diet gave least detectable effects of 0·40 (MAF = 0·05) and 0·18 (MAF = 0·45). Finally, considering the SNP–diet interaction analyses, the least detectable effects were 0·83 (MAF = 0·05) and 0·33 (MAF = 0·45), respectively. All power calculations were performed using QUANTO, version 1.2.4 (May 2009 (http://hydra.usc.edu/gxe/)).

Bonferroni correction was used to adjust for multiple testing, concerning the 240 SNPs in genes presumed to be involved in lipid metabolism, in practice corresponding to an uncorrected significance level of $\alpha = 2\cdot1 \times 10^{-4}$ (= 0·05/240) at baseline/after the LED, and $\alpha = 6\cdot9 \times 10^{-5}$ (= 0·05/(3 × 240)) for main and interaction effects during the *ad libitum* diet period, accounting for SNP main effects, SNP–protein and SNP–GI interaction effects, when testing against a corrected α-level of 0·05. Analyses were performed using Stata 9·2/11·2 (StataCorp LP, 2007/2011).

Results

The characteristics of all participants at inclusion, after the 8-week LED period and after the 6-month *ad libitum* weight maintenance diet are summarised in Table 1. SNPs in lipid metabolism-related genes, with the strongest associations with blood lipids (corresponding to P values <0·001) after the interaction with dietary protein and GI, are presented in Table 2.

None of the SNPs in the lipid metabolism-related genes was found to modify TC, LDL-C, HDL-C or TAG after the 8-week LED, independent of weight loss (Table S3(A)–(D), available online).

Table 1. Characteristics of the participants included in the analyses for baseline, 8 weeks on the low-energy diet and 6-month *ad libitum* weight maintenance diet
(Mean values, standard deviations and number of observations)

| | Baseline | | | Weight loss | | | Weight maintenance | | | | | | | | | | | | | | | |
| | | | | | | | LP/LGI | | | LP/HGI | | | HP/LGI | | | HP/HGI | | | Control | | |
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
Sex (% women)	841	65	–	749	65	–	102	70	–	93	67	–	121	64	–	102	61	–	109	69	–
Age (years)	841	41	6	749	41	6	102	41	6	93	41	6	121	42	7	102	41	6	109	42	7
BMI (kg/m²)	841	34·5	4·9	749	30·6	4·5	102	30·4	4·4	93	30·1	4·2	121	30·6	4·3	100	30·4	4·5	109	30·4	4·5
WC (cm)	830	107·4	13·2	729	97·8	12·3	99	96·9	11·9	89	96·6	10·9	116	96·7	11·8	98	97·2	12·8	108	96·9	12·5
Weight (kg)	841	100·0	17·7	749	89·1	15·9	102	88·5	15·9	93	86·7	14·1	121	88·6	15·6	100	89·2	17·4	109	87·9	16·0
ΔWeight	–	–	–	749	−11·1	3·5	102	0·5	4·9	93	1·9	4·8	121	−0·5	6·5	100	0·6	4·7	109	1·0	4·7
TC (mmol/l)	840	4·9	1·0	737	4·3	0·9	98	4·2	0·9	89	4·1	0·9	115	4·2	0·9	101	4·1	0·9	108	4·1	0·9
ΔTC	–	–	–	732	−0·7	0·8	98	0·7	0·7	89	0·8	0·9	115	0·8	0·8	101	0·6	0·7	108	0·8	0·8
LDL-C (mmol/l)	837	3·1	0·9	733	2·6	0·8	97	2·5	0·8	88	2·4	0·8	116	2·5	0·8	101	2·5	0·8	109	2·5	0·7
ΔLDL-C	–	–	–	728	−0·5	0·6	97	0·4	0·6	88	0·6	0·8	116	0·5	0·7	101	0·4	0·6	109	0·5	0·7
HDL-C (mmol/l)	841	1·2	0·3	737	1·2	0·3	98	1·1	0·3	89	1·2	0·3	115	1·2	0·3	102	1·1	0·3	109	1·2	0·3
ΔHDL-C	–	–	–	734	−0·1	0·2	98	0·2	0·2	89	0·2	0·3	115	0·2	0·2	102	0·2	0·2	109	0·2	0·2
TAG (mmol/l)	831	1·4	0·6	729	1·1	0·5	96	1·1	0·5	87	1·1	0·5	115	1·1	0·5	100	1·0	0·4	107	1·0	0·4
ΔTAG	–	–	–	720	−0·3	0·6	96	0·1	0·5	87	0·2	0·5	115	0·2	0·5	100	0·2	0·4	107	0·2	0·4

LP, low dietary protein; LGI, low glycaemic index; HGI, high glycaemic index; HP, high dietary protein; WC, waist circumference; Δ, change during the low-energy diet and during the 6-month *ad libitum* weight maintenance diet; TC, total cholesterol; LDL-C, LDL-cholesterol; HDL-C, HDL-cholesterol.

Table 2. Associations of SNPs in the lipid metabolism genes with blood lipids after the interaction with dietary protein and glycaemic index, during the 6-month *ad libitum* diet*

(Regression coefficients, 95 % confidence intervals and number of observations for the minor allele)

Gene†	SNPs	Lipid	n	β (mmol/l per allele/dietary unit)	95 % CI	P	$P_{Bonferroni}$
SNP × protein							
LPIN1	rs4315495	TAG	486	−0·26	−0·38, −0·14	0·000043	0·031
ATF6	rs1394070	TAG	486	0·33	0·14, 0·51	0·00045	0·324
SNP × GI							
LPIN2	rs3934427	TC	480	0·56	0·24, 0·87	0·00052	0·374
LPIN2	rs3934427	LDL-C	481	0·46	0·19, 0·73	0·00079	0·569
FABP4	rs16909192	TAG	486	−0·29	−0·46, −0·12	0·00085	0·612

P, uncorrected P value; $P_{Bonferroni}$, Bonferroni-corrected P value; *LPIN1*, lipin 1; *ATF6*, activating transcription factor 6; *LPIN2*, lipin 2; TC, total cholesterol; LDL-C, LDL-cholesterol; *FABP4*, fatty acid-binding protein 4.
* SNP–dietary protein and SNP–glycaemic index associations with blood lipids are estimated by the multiple linear regressions.
† The gene denotes the locus with the closest proximity to the SNP.

After the 6-month *ad libitum* weight maintenance diet, a gene–dietary protein interaction effect on TAG was identified for *LPIN1* rs4315495 with a decrease in TAG of −0·26 mmol/l per A-allele/protein unit (95 % CI −0·38, −0·14, $P=0.000043$; Fig. 1 and Table 2). We did not identify any other significant associations when correcting for multiple testing for SNP main effects (Table S4(A)–(D), available online) or diet interaction effects with dietary protein or GI after the 6-month *ad libitum* weight maintenance diet (Table 2; Tables S5(A)–(D) and S6(A)–(D), available online).

We also examined the associations between SNPs in the lipid metabolism-related genes and baseline blood lipid profile. We identified the associations between *LPL* rs328 and HDL-C with an increase of 0·09 mmol/l per G-allele (95 % CI 0·05, 0·13, $P=0.000044$; Table S2(C), available online), *PPARGC1A* rs10002521 and TC with an increase of 0·19 mmol/l per G-allele (95 % CI 0·09, 0·29, $P=0.00013$; Table S2(A), available online), and *FABP1* rs894194 and TAG with an increase of 0·13 mmol/l per G-allele (95 % CI 0·06, 0·20, $P=0.00018$; Table S2(D), available online). No other SNPs in the lipid metabolism pathways were associated with baseline lipid profile after adjusting for multiple testing (Table S2(A)–(D), available online).

Discussion

In the present randomised dietary intervention study with statistically significant separation of the LP and HP intake groups and the LGI and HGI groups[20], and repeated sampling providing detailed phenotypic characterisation of
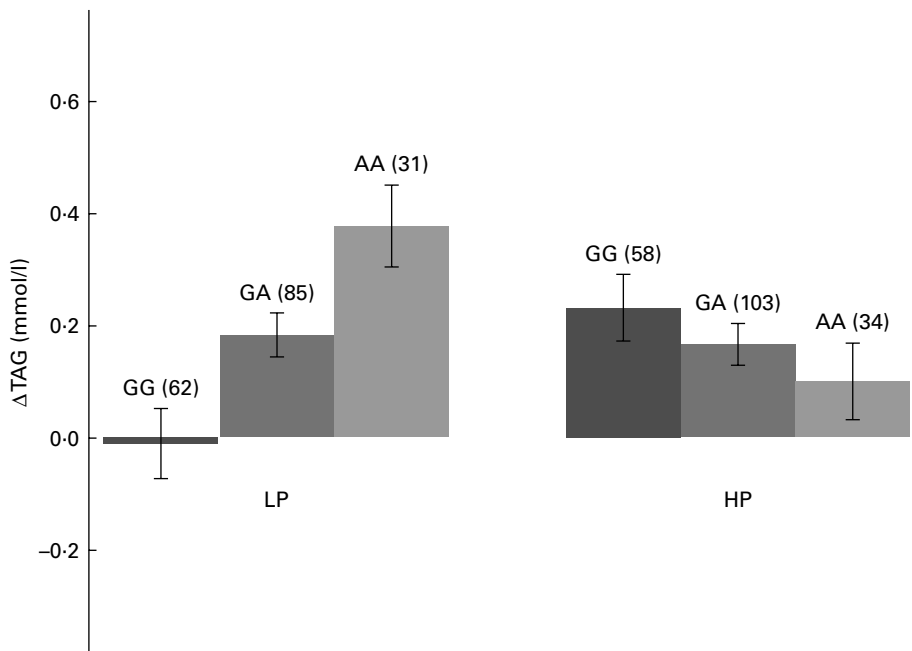

Fig. 1. Effect of the interaction between lipin 1 (*LPIN1*) rs4315495 and dietary protein on TAG during the 6-month *ad libitum* weight maintenance diet. Values are mean changes per genotype for low-protein (LP) *v.* high-protein diets (HP), with standard errors represented by vertical bars. The results for the two HP diets and for the two LP diets are pooled, combining the diets with a low/high glycaemic index. Regression analyses showed a significant interaction between SNP and dietary protein with a decrease in TAG concentration of −0·26 mmol/l per A-allele/protein unit (95 % CI −0·38, −0·14, $P=0.000043$). Outside each bar the genotype is listed, followed by the number of observations. Δ, Change during the 6-month weight maintenance diet.

all participants, we identified an association with plasma TAG for an interaction between *LPIN1* rs4315495 and dietary protein after the *ad libitum* weight maintenance diet. LPIN1 acts as a phosphatidate phosphatase in TAG synthesis[36], and *LPIN1* SNPs have previously been associated with obesity-related phenotypes, insulin sensitivity and the metabolic syndrome[47,48]. Enhanced *LPIN1* expression in transgenic mice promotes increased lipid storage in the adipose tissue and decreased TAG secretion from the liver, whereas *LPIN1*-deficient mice exhibit lipodystrophy and increased hepatic TAG secretion[47]. The present finding indicates that minor allele carriers are more likely to decrease their concentrations of circulating TAG on a high-protein diet than on a low-protein diet. A reduction in the dietary intake of carbohydrates have previously been shown to reduce circulating levels of TAG[49], and participants on the HP diets, in the present study, consumed a lower amount of carbohydrate than participants on the LP diets, which supports this finding.

LPIN1 is located on chromosome 2p25.1, and rs4315495 is located in intron 1. In these analyses, the SNP was not in high LD with any of the other *LPIN1* SNPs ($r^2 < 0.5$), nor were the other fifteen *LPIN1* SNPs associated with changes in TAG by the interaction with dietary protein. The function of rs4315495 is not known, but it is in high LD (r^2 0.87) with rs13412852. The rs13412852 is also an intronic SNP, which has previously been associated with BMI[50] and the severity of non-alcoholic fatty liver disease in children[51]. The association with obesity- and lipid-related phenotypes observed for these two linked SNPs could indicate that they could be linked to another SNP with functional effect or work by affecting as yet unidentified regulatory regions in the loci. Expression of *LPIN1* is regulated by adipocyte differentiation factors, PPARGC1A, sterol regulatory element-binding protein 1, TNF and, possibly, LPIN2[47]. SNPs in *PPARGC1A*, *TNF* and *LPIN2* were also included in the present analyses, but no interactions with dietary protein for the modification of TAG were identified for any of these (Table S4(D), available online).

We did not identify any SNP main effects on blood lipids after the 8-week LED. Previous human intervention studies of gene–diet interaction effects on blood lipids have shown that among overweight individuals, SNPs in *CD36* modified HDL-C and LDL-C by an interaction with a LED[52], and TAG and HDL-C by an interaction with fish oil supplements[53], and SNPs in *MTTP* modified TC and TAG by an interaction with the Mediterranean diet[54]. In addition, several SNPs in genes in lipid metabolism pathways have been shown to modulate blood lipids in response to interventions with varying amounts and types of dietary fat[2,55] and to modulate postprandial blood lipid levels in response to high-fat test meals[56,57]. No studies have addressed interactions between SNPs and dietary protein or the GI in relation to blood lipids.

Among the selected lipid metabolism genes, only *LPL* and *MLXIPL* have previously been shown to be associated with lipid concentrations in GWAS[15]. We identified a significant association of *LPL* rs328 with baseline HDL-C (Table S2(C), available online). *LPL* encodes the enzyme LPL with the primary function to hydrolyse TAG in circulating lipoproteins[38]. The present finding is consistent with previous reports in terms of effect size, risk allele and MAF[6,11], and generally in line with GWAS that have found associations between SNPs in *LPL* and lipoprotein metabolism, including associations between several *LPL* SNPs and HDL-C[4,5,10,13], with the reported SNPs rs17482753[12], rs12678919[7,14,15] and rs10503669[9,14] in complete LD with rs328.

In GWAS, *MLXIPL* have been associated with LDL-C[5], HDL-C[15] and TAG[4,6–8,11,15,58]. *MLXIPL* encodes the carbohydrate-responsive element-binding protein, which is an essential transcription factor for lipogenesis[39]. The *MLXIPL* SNPs included in the present study are different from the ones that were found to be associated with lipid profile in these GWAS, and LD was not strong (pairwise $r^2 < 0.8$ for all SNPs). However, the present analyses do suggest that *MLXIPL* rs1051921 could be associated with lower baseline levels of TAG (Table S2(D), available online).

Baseline associations were also identified between *PPARGC1A* rs10002521 and TC and between *FABP1* rs894194 and TAG. *FABP1* encodes the liver fatty acid-binding protein that plays a key role in fatty acid metabolism, and variation in this gene has previously been associated with circulating TAG and LDL-C in human subjects[32]. *PPARGC1A* encodes the protein PPARGC1A with a regulatory role in fatty acid metabolism and mitochondrial function[44]. The identified association for *PPARGC1A* has not previously been shown, but variants in *PPARGC1A* have previously been associated with type 2 diabetes[59].

The present study is limited by the relatively low number of participants in the DiOGenes study. While high for an intervention study, it is low in terms of conducting this type of genetic analyses. The candidate gene approach, although with a coverage of the genetic variation of the loci within 5 kb up/downstream and with a tagSNP disequilibrium limit of 0.7–0.8, limits the study particularly in detecting baseline associations, but also in identifying SNP–diet interactions, and the selected candidate genes in the study do not represent all known lipid-associated genes, nor do the SNPs include all of the recently identified lipid-associated SNPs[15].

In conclusion, in the present analyses of 240 SNPs in presumed nutrient-sensitive lipid metabolism genes among overweight and obese European adults, we identified an interaction between *LPIN1* rs4315495 and dietary protein that resulted in a decrease in TAG concentration for minor allele carriers on the high-protein weight maintenance diet. Adjusting for multiple testing, no other effects of SNPs or SNP–diet (protein content or GI) interactions on blood lipid profile were detected after weight loss or after the 6-month *ad libitum* weight maintenance diet.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S0007114512006058

Acknowledgements

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