# *FADS2* genotype regulates delta-6 desaturase activity and inflammation in human adipose tissue[S]

**Maija Vaittinen,* Paula Walle,* Emmi Kuosmanen,* Ville Männistö,† Pirjo Käkelä,§ Jyrki Ågren,** Ursula Schwab,*,†† and Jussi Pihlajamäki[1,*,††]**

Institutes of Public Health and Clinical Nutrition* and Biomedicine,** University of Eastern Finland, Kuopio, Finland; Departments of Medicine† and Surgery,§ University of Eastern Finland and Kuopio University Hospital, Kuopio, Finland; and Clinical Nutrition and Obesity Center,†† Kuopio University Hospital, Kuopio, Finland

**Abstract Obesity is associated with disturbed lipid metabolism and low-grade inflammation in tissues. The aim of this study was to investigate the association between FA metabolism and adipose tissue (AT) inflammation in the Kuopio Obesity Surgery study. We investigated the association of surgery-induced weight loss and FA desaturase (*FADS*)*1/2* genotypes with serum and AT FA profile and with AT inflammation, measured as interleukin (*IL*)*-1β* and NFκB pathway gene expression, in order to find potential gene-environment interactions. We demonstrated an association between serum levels of saturated and polyunsaturated n-6 FAs, and estimated enzyme activities of *FADS1/2* genes with *IL-1β* expression in AT both at baseline and at follow-up. Variation in the *FADS1/2* genes associated with *IL-1β* and NFκB pathway gene expression in SAT after weight reduction, but not at baseline. In addition, the FA composition in subcutaneous and visceral fat correlated with serum FAs, and the associations between serum PUFAs and estimated D6D enzyme activity with AT inflammation were also replicated with corresponding AT FAs and AT inflammation.[JLR] We conclude that the polymorphism in *FADS1/2* genes associates with FA metabolism and AT inflammation, leading to an interaction between weight loss and *FADS1/2* genes in the regulation of AT inflammation.—**Vaittinen, M., P. Walle, E. Kuosmanen, V. Männistö, P. Käkelä, J. Ågren, U. Schwab, and J. Pihlajamäki. ***FADS2* genotype regulates delta-6 desaturase activity and inflammation in human adipose tissue.** ***J. Lipid Res.*** **2016.** 57: **56–65.**

**Supplementary key words** fatty acid • fatty acid/desaturases • fatty acid/metabolism • gene expression • genetics • obesity • fatty acid desaturase 1

Low-grade inflammation is associated with metabolic diseases, such as type 2 diabetes, cardiovascular diseases, cancer, and nonalcoholic fatty liver disease (1, 2). It is linked to energy excess in diet, i.e., obesity, and also to the quality of dietary fats, particularly to diets rich in saturated FAs (SFAs) (2, 3). Several studies demonstrate that SFAs promote inflammation through toll-like receptor 4 (TLR4) and activation of the, so called, inflammasome complex, which is responsible for the regulation of interleukin (IL)-1β production (1, 4, 5). In turn, an increased production of IL-1β has a major role in adipose tissue (AT) inflammation by stimulating macrophage infiltration into AT (6, 7).

The role of PUFAs in AT inflammation is more complex. Overall, n-3 PUFAs are considered to be anti-inflammatory (5). However, n-6 PUFAs are related to pro-inflammatory events, most likely due to the fact that arachidonic acid (AA), produced from linoleic acid (LA), is a major substrate for eicosanoids that regulate inflammatory responses (5). The main FA desaturase (FADS) enzymes regulating PUFA metabolism are delta-5 desaturase (D5D) and delta-6 desaturase (D6D), encoded by the *FADS1* and *FADS2* genes, respectively (8). Genetic variations in the *FADS* genes and isoforms of these genes have been associated with plasma PUFA concentrations and pro-inflammatory markers, diabetes, and metabolic syndrome, indicating that activity of these enzymes may modify the effect of dietary fat on tissue inflammation (3, 9, 10).

To investigate the association between FA metabolism and tissue inflammation, we measured the serum and AT FA profile in different lipid fractions and *IL-1β* gene expression in subcutaneous AT (SAT), visceral AT (VAT),

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Abbreviations: AA, arachidonic acid; AT, adipose tissue; D5D, delta-5 desaturase; D6D, delta-6 desaturase; FADS, FA desaturase; GLA, γ-linolenic acid; IL, interleukin; IR, insulin resistance; KOBS, Kuopio Obesity Surgery; LA, linoleic acid; NFκB, nuclear factor κ B; PBMC, peripheral blood mononuclear cell; SAT, subcutaneous adipose tissue; SCD, stearoyl-CoA desaturase; SFA, saturated FA; TLR4, toll-like receptor 4; TREx, TruSeq targeted RNA expression; VAT, visceral adipose tissue; VLC, very low calorie.

[1]To whom correspondence should be addressed.
e-mail: jussi.pihlajamaki@uef.fi

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liver, and peripheral blood mononuclear cells (PBMCs) after a standardized 4–5 week very low calorie (VLC) diet period in 89 obese individuals undergoing obesity surgery. Furthermore, the effect of surgery-induced weight loss and genotypes of the *FADS1* and *FADS2* on serum and AT FA profile and AT inflammation, measured as *IL-1β* gene expression and using TruSeq Targeted RNA Expression NFκB pathway gene panel, was investigated in order to find potential gene-environment interactions.

## MATERIAL AND METHODS

### Study population

The Kuopio Obesity Surgery (KOBS) study is an ongoing study, with 89 individuals (age 46.3 ± 8.8 years, BMI 44.9 ± 6.3 kg/m$^2$) currently included in it (**Table 1**) (11). Clinical parameters were assessed prior to the gastric bypass surgery (at baseline, n = 89) and at 1 year after surgery (follow-up, n = 64). Biopsies from liver, SAT and VAT, and PBMCs were collected from the participants at the time of surgery. In addition, SAT and liver biopsies were taken 1 year after the surgery (11 individuals available for follow-up liver biopsy). Blood samples were drawn after a 12 h overnight fast, both at baseline and follow-up. All participants were guided to follow a VLC diet for 4–5 weeks before the surgery with the aim to lose weight (10%) before the operation. A time point after the VLC diet is considered as baseline. For the purposes of this study, it is essential to note that diet was standardized with the aim to reach a caloric intake of 800–1,000 kcal using primarily commercial products for the VLC diet. Weight loss in this study during the 1 year follow-up was −24 ± 9% (Table 1). The study protocol was approved by the Ethics Committee of Northern Savo Hospital District and carried out in accordance with the Helsinki Declaration. Informed written consent was obtained from all participants.

### Biochemical measurements

Plasma glucose, insulin, and serum lipid (total cholesterol, HDL-cholesterol, and TGs) levels were measured from fasting venous blood samples. Plasma glucose was measured by enzymatic hexokinase photometric assay (Konelab Systems reagents; Thermo Fischer Scientific, Vantaa, Finland). Insulin was determined by immunoassay (ADVIA Centaur Insulin IRI, number 02230141; Siemens Medical Solutions Diagnostics, Tarrytown, NY). An enzymatic colorimetric method (Wako NEFA C test kit; Wako Chemicals, Neuss, Germany) was used for serum FFA measurement.

### AT gene expression

All samples for gene expression analysis were immediately frozen in liquid nitrogen. Total RNA from AT was extracted using Tri-Reagent (Applied Biosystems, Foster City, CA) and reverse-transcribed using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems) according to the manufacturer's protocol. Quantitative real-time PCR was carried out with the Applied Biosystems 7500 real-time PCR system using KAPA SYBR FAST qPCR Universal Master Mix (Kapa Biosystems, Woburn, MA). The primer sequences for *IL-1β* are forward 5′-CAGCTACGAATCTCCGACCA and reverse 5′-TCCATGGCCACAACAACTGA and for *RPLP0*, which was used as an endogenous control gene, forward 5′-GGCGACCTGGAAGTCCAACT and reverse 5′-CCATCAGCACCACAGCCTTC.

### TruSeq targeted RNA expression

A fixed NFκB pathway gene panel (Illumina, San Diego, CA) was used for measuring gene expression levels in human SAT (n = 86) before and 1 year after surgery using the MiSeq system (Illumina) according to instructions provided by the manufacturer. Total RNA (50 ng) from SAT was reverse-transcribed using ProtoScript II reverse transcriptase (New England BioLabs). Oligo pool targeted regions of interest were hybridized to cDNA. Next, hybridized cDNA was extended by DNA polymerase followed by ligation using DNA ligase. The extension-ligation products were amplified with PCR and AMPure XP beads (Beckman Coulter) and used to clean up the PCR products. Equal volumes of the products were pooled together and quantitated with DNA 1000 chip (Agilent Technologies, Wilmington, DE). Finally, the pooled sample was diluted and denatured and sequenced with MiSeq.

### Assessment of FA composition

FA composition in serum and AT was analyzed according to previously described methods (12, 13). In short, the serum samples were extracted with chloroform-methanol (2:1) and the different lipid fractions, cholesteryl ester, TG, and phospholipid, were separated by solid phase extraction with an aminopropyl column. The AT samples were pulverized with liquid nitrogen and approximately 40 mg of AT was extracted with chloroform-methanol (2:1), as described above. Serum and AT FAs were transmethylated with 14% boron trifluoride in methanol and were analyzed by a 7890A gas chromatograph (Agilent Technologies) equipped with a 25 m FFAP column. Cholesteryl nonadecanoate (Nu Chek Prep, Inc., Elysian, MA), trinonadecanoin, and phosphatidylcholine dinonadecanoyl (Larodan Fine Chemicals, Malmö, Sweden) served as internal standards. Enzyme activities in different lipid fractions were estimated as product-to-precursor ratios of individual FAs as follows: stearoyl-CoA desaturase (SCD) = 16:1 n-7/16:0 or 18:1 n-9/18:0, elongase = 18:0/16:0 or 18:1 n-7/16:1 n-7, D5D = 20:4 n-6/20:3 n-6, D6D = 18:3 n-6/18:2 n-6, and de novo lipogenesis = 16:0/18:2 n-6. The terms D5D and D6D are used for the estimated enzyme activities in serum and AT, and *FADS1* and *FADS2* in italic type are used for the genes.

TABLE 1. Clinical characteristics at baseline and at 1 year follow-up in the KOBS study

| | Baseline (n = 89) | 1 Year (n = 64) | *P* |
|---|---|---|---|
| Age (years) | 46.31 ± 8.77 | 46.85 ± 8.60 | — |
| BMI (kg/m$^2$) | 44.87 ± 6.32 | 34.45 ± 5.67 | $9 \times 10^{-30}$ |
| Total cholesterol (mmol/l) | 4.48 ± 1.08 | 4.45 ± 1.02 | $3 \times 10^{-01}$ |
| HDL-cholesterol (mmol/l) | 1.10 ± 0.30 | 1.42 ± 0.39 | $1 \times 10^{-16}$ |
| LDL-cholesterol (mmol/l) | 2.68 ± 0.96 | 2.50 ± 0.90 | $9 \times 10^{-03}$ |
| TGs (mmol/l) | 1.58 ± 0.70 | 1.18 ± 0.48 | $3 \times 10^{-09}$ |
| Fasting glucose (mmol/l) | 6.58 ± 1.65 | 5.51 ± 0.90 | $5 \times 10^{-07}$ |
| Fasting insulin (mU/l) | 21.91 ± 23.08 | 11.81 ± 11.71 | $2 \times 10^{-08}$ |

Values are presented as mean ± SD, paired samples *t*-test.

### Genotype analyses

The variants, rs174547 (*FADS1*) and rs174616 (*FADS2*), were genotyped from 154 DNA samples of the KOBS study using the TaqMan SNP genotyping assay (Applied Biosystems) according to the manufacturer's protocol. These SNPs were chosen for the current analysis because of their previous association with PUFA levels and desaturase activities (14–17).

### Statistical analysis

Statistical analyses were conducted with SPSS software (version 19; SPSS Inc., Chicago, IL). Paired samples *t*-test was used to compare the baseline and follow-up data and ANOVA was used for the comparison between genotype groups. The correlation between variables was analyzed using Spearman's nonparametric correlation. The Hardy-Weinberg equilibrium and genotype distribution among study groups were analyzed with the $\chi^2$-test. Genetic associations with continuous variables were analyzed with general linear models univariate ANOVA and Bonferroni's multiple correction was used when there was a significant association in order to find differences between genotype groups. For the TruSeq targeted RNA expression (TREx) analysis, the expression levels for each gene per sample in the NFκB gene panel were normalized based on the total number of aligned reads of the corresponding sample and the results are shown as percentage of total transcript reads. The results of the TREx analysis were analyzed for the deviation of Spearman's correlations between the expression of individual genes and respective FA parameters from the null hypothesis (correlation coefficient = 0) using one sample *t*-test. Paired samples (Spearman's correlation coefficient for individual genes at baseline and follow-up) *t*-test was used to compare the baseline and follow-up Spearman correlation coefficients. Descriptive statistics are presented as mean ± SD. False discovery rate-adjusted *P* value ($P < 0.05$) using the Benjamini and Hochberg method was considered statistically significant in the TREx analysis.

## RESULTS

### Characteristics

BMI, levels of serum total TGs, LDL-cholesterol, fasting plasma glucose, and insulin decreased, and the level of HDL-cholesterol increased, in response to surgery ($P < 0.05$, Table 1), as previously published in the same cohort (18, 19).

### *IL-1β* expression is decreased after surgery-induced weight loss in several human tissues

First, to set up the model in which we investigated the relationship between tissue inflammation and FA metabolism, we measured *IL-1β* mRNA expression in AT, liver, and PBMCs before and after surgery. The expression was decreased in SAT ($P = 0.035$) and PBMCs ($P = 2.25 \times 10^{-8}$) in response to surgery (**Table 2**). In the liver, there was a tendency for reduced *IL-1β* expression after weight loss ($P = 0.343$). However, the difference was not significant, probably due to the low number of liver samples in the follow-up (n = 11).

### Serum FA profile associates with *IL-1β* expression in AT

In the analysis of serum and AT FA composition in different lipid fractions, we concentrated on the TG fraction due to the fact that the FA profile in this fraction reflects both the diet and endogenous FA metabolism (20). Furthermore, the strongest correlations between serum FA composition and tissue *IL-1β* expression were observed in the TG fraction. Correlations between tissue *IL-1β* expression and FA composition in serum cholesteryl ester, phospholipid, and AT TG fractions are shown in supplementary Table 1.

There was a strong positive correlation between the proportion of serum SFA in the TG fraction and *IL-1β* expression in VAT (**Fig. 1B**). For individual SFAs in the TG fraction, positive correlations of myristic acid and stearic acid were higher with *IL-1β* expression in VAT than with *IL-1β* expression in SAT (**Table 3**). Total PUFA proportion in the TG fraction correlated negatively with *IL-1β* expression in VAT, but not in SAT (Fig. 1A, B). This could be explained by negative correlation of LA, a substrate for D6D activity, with *IL-1β* expression in VAT. On the other hand, the correlation of γ-linolenic acid (GLA), a product of D6D activity, was positive with *IL-1β* expression both in SAT and in VAT ($P = 0.012$ and $P = 0.001$, respectively). In fact, GLA was the only one of the individual FAs that correlated significantly with *IL-1β* expression in SAT at the follow-up (Table 3). The proportion of serum MUFAs in the TG fraction correlated negatively with *IL-1β* expression in SAT, but not in VAT (Fig. 1A, B). Among the MUFAs in the TG fraction (Table 3), oleic acid correlated negatively with *IL-1β* expression in SAT and VAT ($P < 0.05$), and eicosenoic acid correlated positively with *IL-1β* expression in SAT at baseline ($P < 0.01$). There were no correlations between serum FAs in different lipid fractions and *IL-1β* expression in the liver at baseline (supplementary Table 1) or PBMCs (not shown). Taken together, the correlations of serum FA composition in the TG fraction with *IL-1β* expression in tissues was stronger in VAT than in SAT, and there were few correlations between serum FA composition in different lipid fractions and *IL-1β* expression in liver or PBMCs (supplementary Table 1).

### Estimated D6D enzyme activity in serum TG fraction correlates with *IL-1β* expression in SAT

Next we investigated whether the correlations between serum FA composition and AT inflammation could be

TABLE 2. *IL-1β* mRNA expression in different tissues at baseline and follow-up in the KOBS study

| *IL-1β* Expression (AU) | Baseline | Follow-up | *P* |
|---|---|---|---|
| PBMC (n = 56) | 0.00423 ± 0.0026 | 0.00209 ± 0.0016 | $2 \times 10^{-8}$ |
| AT (n = 65) | 0.00209 ± 0.0031 | 0.00123 ± 0.0014 | 0.035 |
| Liver (n = 11) | 0.00143 ± 0.0018 | 0.00089 ± 0.0005 | 0.343 |

Values are presented as mean ± SD, paired samples *t*-test. AU, arbitrary unit.

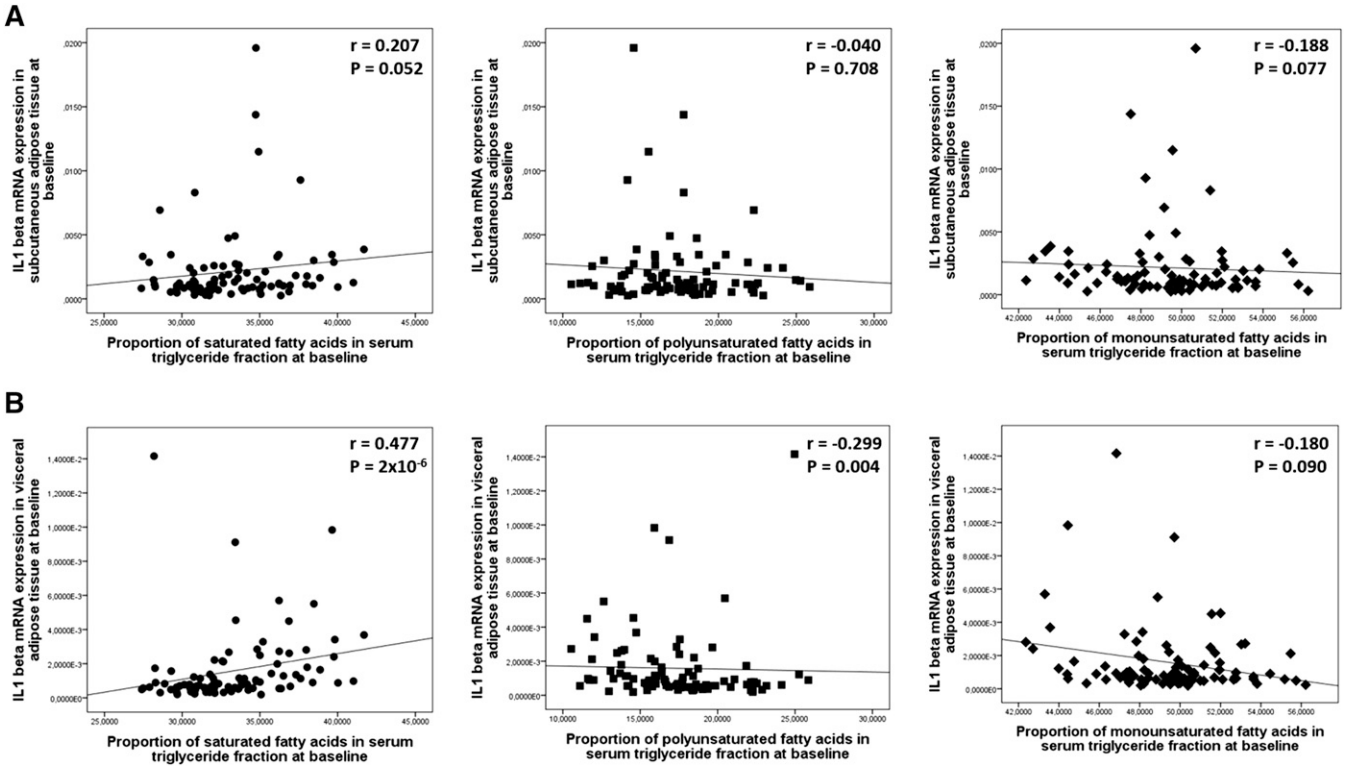


**Fig. 1.** Scatterplots demonstrating correlations of *IL-1β* gene expression in SAT (n = 89) (A) and VAT (n = 90) (B) with the proportions (as mole percent) of SFAs (black circles), PUFAs (black squares), and MUFAs (black diamonds) in the serum TG fraction at baseline of the KOBS study.

linked to changes with the activity indexes of the enzymes regulating endogenous FA metabolism. In general, the correlations of the enzyme activity indexes in the TG fraction with *IL-1β* expression at baseline were similar in both AT depots (**Fig. 2**). The only exception was the de novo lipogenesis enzyme activity, which correlated positively with *IL-1β* expression in VAT, but not in SAT (supplementary Table 2). Of the other desaturase enzyme activity indexes, the correlations of D5D and SCD activity on 18 carbon FAs in the TG fraction with *IL-1β* expression were negative at baseline, but not at follow-up (Fig. 2A–C). The estimated D6D enzyme activity in the TG fraction was the only index that correlated positively with *IL-1β* expression both in VAT and SAT at baseline, and with *IL-1β* expression in SAT at follow-up (Fig. 2A, C). There were no correlations of SCD activity on 16 carbon FAs with *IL-1β* expression (supplementary Table 2). In addition, *IL-1β* expression in both AT depots correlated positively with elongase enzyme activity on SFAs (18:0/16:0), but there was no correlation with the elongase activity on MUFAs (18:1n-7/16:1n-7, supplementary Table 2). The correlations of enzyme activity indexes with *IL-1β* expression in liver and PBMCs are shown in supplementary Table 2.

### Genetic variations in *FADS1* and *FADS2* genes associate with D5D and D6D activities and *IL-1β* expression in AT

Because the estimated D5D and D6D enzyme activities in the TG fraction correlated with AT inflammation, we next investigated whether genetic variations in *FADS1* and *FADS2* genes associate with serum FA profile, as published before (14–17), and with *IL-1β* expression in AT. The SNPs, rs174616 (*FADS2*) and rs174547 (*FADS1*), were in Hardy-Weinberg equilibrium (0.277, 0.591), and in linkage disequilibrium ($r^2 = 0.33$, D'= 0.70), as known due the existence of these genes in the same gene cluster (17). There were no associations of these SNPs with anthropometric measures, glucose and insulin concentrations, and with concentrations of serum TGs, or total, LDL-, or HDL-cholesterol at baseline or follow-up, despite a known association with serum lipids in the genome-wide association studies (14, 17). The lack of associations with serum lipids may be explained by a reduced statistical power compared with genome-wide association studies. However, we could clearly demonstrate an association of the *FADS1* and *FADS2* SNPs with estimated D5D and D6D enzyme activity indexes at baseline and follow-up ($P < 0.006$, **Fig. 3A, B**). The estimated D6D enzyme activity in the serum TG fraction increased after weight loss (0.022 ± 0.011 vs. 0.025 ± 0.016, $P < 0.001$), and associated with *FADS2* genotype so that subjects with major allele (G) were associated with increased enzyme activity of D6D after weight loss (0.024 ± 0.012 vs. 0.032 ± 0.018, $P < 0.001$). In addition, the change of D6D enzyme activity was associated both with *FADS2* ($P = 0.001$) and *FADS1* ($P = 4.0 \times 10^{-4}$) genotypes. The estimated D5D enzyme activity was not changed after weight loss (5.540 ± 2.338 vs. 5.673 ± 1.946, $P = 0.482$).

The most important finding of the genetic analysis was that both variants, rs174547 (*FADS1*) and rs174616

TABLE 3. Spearman's correlation coefficients of *IL-1β* expression in SAT and VAT with the serum FA profile of the TG fraction in the KOBS study

| | Baseline | | Follow-up |
|---|---|---|---|
| FA (mol%) | SAT (n = 89) | VAT (n = 90) | SAT (n = 64) |
| SFA | | | |
| Myristic acid (14:0) | 0.301[b] | 0.453[b] | 0.161 |
| Palmitic acid (16:0) | 0.129 | 0.430[b] | 0.117 |
| Stearic acid (18:0) | 0.377[b] | 0.435[b] | 0.209 |
| MUFA | | | |
| Palmitoleic acid (16:1n-7) | 0.026 | 0.025 | 0.152 |
| Oleic acid (18:1n-9) | −0.223[a] | −0.234[a] | −0.068 |
| Vaccenic acid (18:1n-7) | −0.108 | −0.093 | −0.026 |
| Eicosenoic acid (20:1n-9+11) | 0.324[b] | 0.202 | −0.088 |
| PUFA | | | |
| Total n-6 FA | −0.072 | −0.314[b] | −0.140 |
| LA (18:2n-6) | −0.038 | −0.305[b] | −0.159 |
| GLA (18:3n-6) | 0.267[a] | 0.344[b] | 0.284[a] |
| Dihomo-γ-linolenic acid (20:3n-6) | 0.133 | 0.158 | 0.173 |
| AA (20:4n-6) | −0.185 | −0.151 | 0.001 |
| Total n-3 FA | 0.011 | −0.160 | −0.025 |
| α-Linolenic acid (18:3n-3) | 0.148 | 0.026 | −0.094 |
| Eicosapentaenoic acid (20:5n-3) | 0.004 | −0.038 | 0.004 |
| Docosahexaenoic acid (22:6n-3) | −0.052 | −0.148 | −0.040 |

[a] $P < 0.05$.
[b] $P < 0.01$.

(*FADS2*), were associated with *IL-1β* expression in SAT after weight reduction at 1 year follow-up, but not at baseline, indicating that these genes are able to regulate AT inflammation after weight loss-induced reduction in AT inflammation ($P = 0.004$ and $P = 0.031$, respectively, Fig. 3C, D). This association was also significant after adjustment with the change of BMI ($P = 0.003$, $P = 0.032$, respectively). Individuals having major alleles (T or G) of the *FADS1/2* polymorphisms that associate with increased D5D and D6D enzyme activities after weight loss (Fig. 3A, B) continued to also have higher *IL-1β* expression after weight loss, whereas subjects with minor alleles (C or A) tended to decrease *IL-1β* expression after weight loss ($P = 0.135$) (Fig. 3C, D).

### Analysis of the TREx NFκB pathway gene panel

Because our results were based only on the expression of a single gene, *IL-1β*, we proceeded to the analysis of the NFκB pathway gene panel. Gene expression and correlation results are shown in supplementary Tables 3 and 4. We could verify that inflammatory genes had, on average, a positive correlation with serum SFAs of the TG fraction (average Spearman's correlation 0.077 ± 0.125) (**Fig. 4A**) and the estimated D6D enzyme activity index (0.028 ± 0.130) (Fig. 4B), and, on average, a negative correlation with serum PUFAs of the TG fraction (−0.027 ± 0.125) (Fig. 4A) at follow-up, but not at baseline. Importantly, the effect of *FADS1* and *FADS2* genotypes on inflammatory genes (0.044 ± 0.103 and 0.049 ± 0.104, respectively) was observed at follow-up, but not at baseline (Fig. 4C), similarly as observed with an association between the genotypes and *IL-1β* gene expression (Fig. 3C, D). Finally, the average correlation of NFκB pathway genes with SFAs of the TG fraction and *FADS1/2* genotypes at follow-up differed significantly when compared with baseline ($P = 7 \times 10^{-6}$, $P = 0.0125$, and $P = 0.0153$, respectively; Fig. 4A, C).

### FA composition in the TG fraction of AT

FA composition and correlation results in serum and AT TG fractions are shown in supplementary Table 5. The most abundant FAs in the TG fraction of serum and AT depots were 16:0, 18:1n-9, and 18:2n-6, as also shown before (21). Our results showed strong correlations between the FA composition in the TG fraction in serum and both AT depots (supplementary Table 5). We could replicate the association of serum PUFAs and estimated D6D enzyme activity with AT inflammation, measured either with *IL-1β* mRNA expression or using the NFκB gene panel, also when we measured AT FAs instead of serum FAs. Similarly to serum analyses, the estimated D6D enzyme activity in the TG fraction of SAT correlated positively with *IL-1β* expression in SAT at follow-up (supplementary Table 2). Importantly, the results also demonstrated that NFκB pathway-related inflammatory genes in SAT correlated, on average, negatively with PUFA composition in the TG fraction of SAT, both at baseline and at follow-up, similarly as observed with serum FA analyses. Furthermore, expression of NFκB pathway genes correlated negatively with the estimated D5D enzyme activity in the TG fraction of SAT at baseline and positively with estimated D6D enzyme activity in the TG fraction of SAT at follow-up (**Fig. 5**). Finally, subjects with *FADS2* GG genotype tended to have higher estimated D6D enzyme activity in the TG fraction of SAT ($P = 0.096$) and VAT ($P = 0.095$), as was shown with serum analysis. Overall, the observations in AT FAs verified the results with serum FAs that the association of lower levels of PUFAs and higher levels of estimated D6D enzyme activity with AT inflammation is revealed after weight loss.

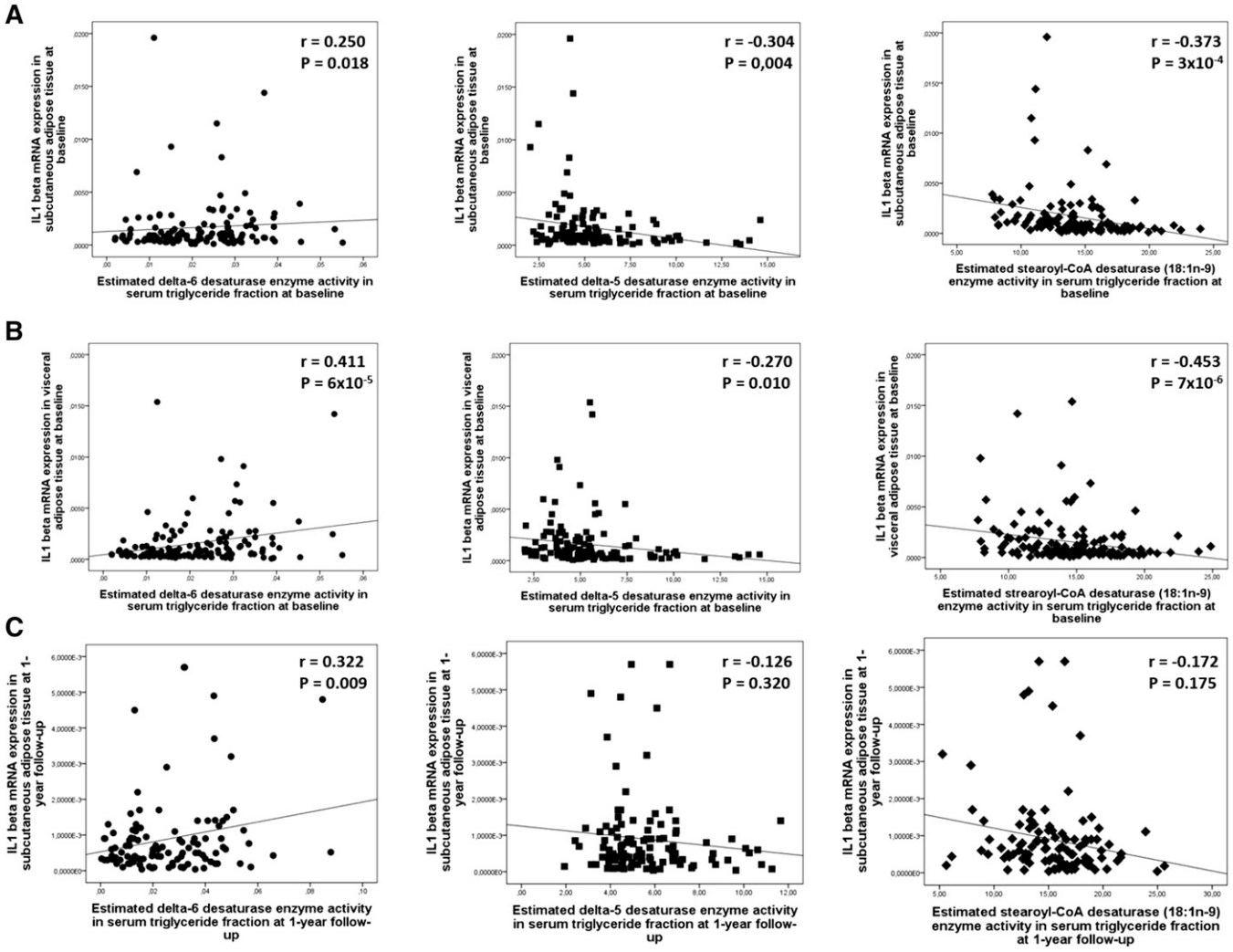


**Fig. 2.** Scatterplots demonstrating correlations of *IL-1β* gene expression in SAT (n = 89) (A), VAT (n = 90) (B) with estimated enzyme activities at baseline, and in SAT (n = 64) (C) at follow-up of the KOBS study. Black circles, D6D (18:3n-6/18:2n-6); black squares, D5D (20:4n-6/20:3n-6); black diamonds, SCD 18:1n-9 (18:1n-9/18:0).

## DISCUSSION

We investigated the association between FA metabolism and tissue inflammation after standardized VLC diet and after a substantial weight loss induced by obesity surgery. As expected, we observed a decrease in *IL-1β* expression in several tissues in response to weight loss (Fig. 1) (18, 19). However, the association between serum FA composition in the TG fraction and *IL-1β* expression were observed both after the standardized diet and after the surgery. This suggests that there is an association between endogenous FA metabolism and *IL-1β* expression in AT. In fact, estimated desaturase enzyme activities, both in serum and SAT, associated with *IL-1β* expression in AT, and *FADS1* and *FADS2* genotypes associated with *IL-1β* expression and expression of genes in the NFκB pathway after follow-up in AT (Figs. 3, 4). All these findings suggest that the genetic variation in the *FADS1/2* genes and the corresponding desaturase enzyme activities are involved in endogenous FA metabolism and AT low-grade inflammation.

The regulation of PUFA-derived pro- and anti-inflammatory eicosanoid levels by D5D and D6D activities are of great interest in the context of AT low-grade inflammation (15). D6D and D5D have been shown to have opposite effects, as shown by an inverse correlation of D5D and a direct correlation of D6D activities with the risk of diabetes and insulin resistance (IR) (22). Recently, it has been shown that a genetic variation in *FADS2* gene, encoding for D6D, was associated with decreased risk of type 2 diabetes (23). Interestingly, the most consistent findings of our study related to the *FADS2* gene and corresponding D6D enzyme activity in the TG fraction, calculated as the ratio of serum 18:3 n-6/18:2 n-6. First, we could show that both serum and SAT D6D activities associate with *IL-1β* expression in several tissues (Fig. 2, supplementary Table 2). Second, subjects who had TT or GG genotypes of *FADS1/2* polymorphism, which associated with higher D5D and D6D enzyme activities, also associated with higher *IL-1β* expression in SAT after weight loss, when compared with other genotypes. However, *IL-1β* expression at baseline was not

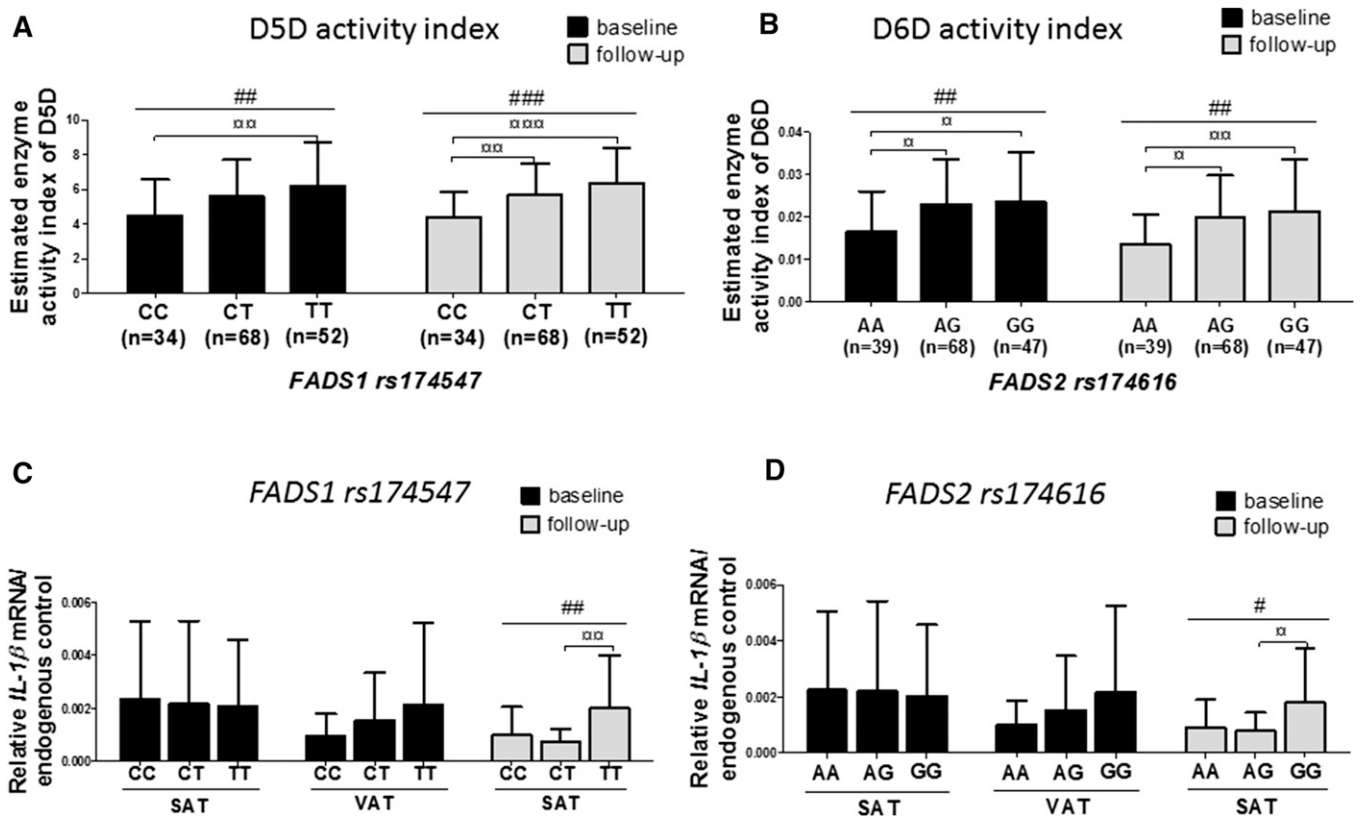


**Fig. 3.** Enzyme activities of D5D (A) and D6D (B), and *IL-1β* expression in SAT and VAT (C, D) according to the *FADS1* and *FADS2* genotypes in the KOBS study. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ (univariate general linear model), ¤$P < 0.05$, ¤¤$P < 0.01$, and ¤¤¤$P < 0.001$ (additive genetic model in univariate general linear model analysis with Bonferroni's correction).

influenced by *FADS1* and *FADS2* genetic variations. This suggests a sustained AT inflammation in subjects with TT or GG genotypes of *FADS1* and *FADS2* genes despite the fact that *IL-1β* expression decreased after weight reduction. This could be explained, in part, by the fact that the change of BMI was smaller in subjects with GG genotype ($P = 0.03$) when compared with AA genotype of the *FADS2*, indicating a possible decreased effect of weight loss on AT low-grade inflammation. It should be noted that D6D, as well as D5D, activities are regulated by insulin (24, 25). Therefore, the effect of the genotype on *IL-1β* expression may have been revealed in our study after the improvement of hyperinsulinemia in response to weight loss. Overall, these findings confirm the significance of the *FADS1* and *FADS2* SNPs in the regulation of diabetes risk and tissue inflammation (22). Furthermore, our results indicate a gene-environment interaction in the regulation of AT *IL-1β* expression by FADSs. As a mechanism, we suggest that genetic variation in *FADS2* that increases the activity of D6D resulting in increased levels of serum AA interacts with the inflammatory/anti-inflammatory effects of the diet. AA, as a precursor for pro-inflammatory eicosanoids, could be further metabolized to produce pro-inflammatory leukotrienes, which are known to activate NFκB to produce *IL-1β* (26).

IL-1β is considered to be a marker of low-grade inflammation, which contributes to development of IR and type 2 diabetes (6, 7). Low-grade inflammation in VAT is more strongly correlated with the metabolic disorders than inflammation in SAT (6, 27), and *IL-1β* expression is shown to be more abundant in VAT than in SAT in obese individuals (7). In line with this, our results showed a more clear relationship between serum FA profile in the TG fraction and *IL-1β* expression in VAT than in SAT, suggesting that the interaction between FA metabolism and *IL-1β* expression is more prominent in VAT. For example, serum total SFAs and individual SFAs (myristic, palmitic, and stearic) in the TG fraction correlated positively with *IL-1β* expression in VAT, but not in SAT. This is further supported by our results, which showed a negative correlation of *IL-1β* expression and SCD activity on 18 carbon FAs and by a positive correlation with elongase (18:0/16:0) activity. Decreased SCD activity is associated with increased SFA accumulation and development of inflammation (15), and elongase (18:0/16:0) is responsible for the production of SFAs by the elongation of palmitic acid to stearic acid (28). Previous studies have shown that SFAs stimulate IL-1β-induced AT inflammation through activation of the TLR4/NFκB pathway and inflammasome complex (29, 30). Recently, it has also been shown that MUFAs can attenuate IL-1β-mediated AT inflammation through AMPK activation (29). In line with this, our results also showed a negative correlation between oleic acid and *IL-1β* expression in both adipose depots at baseline of the KOBS study.

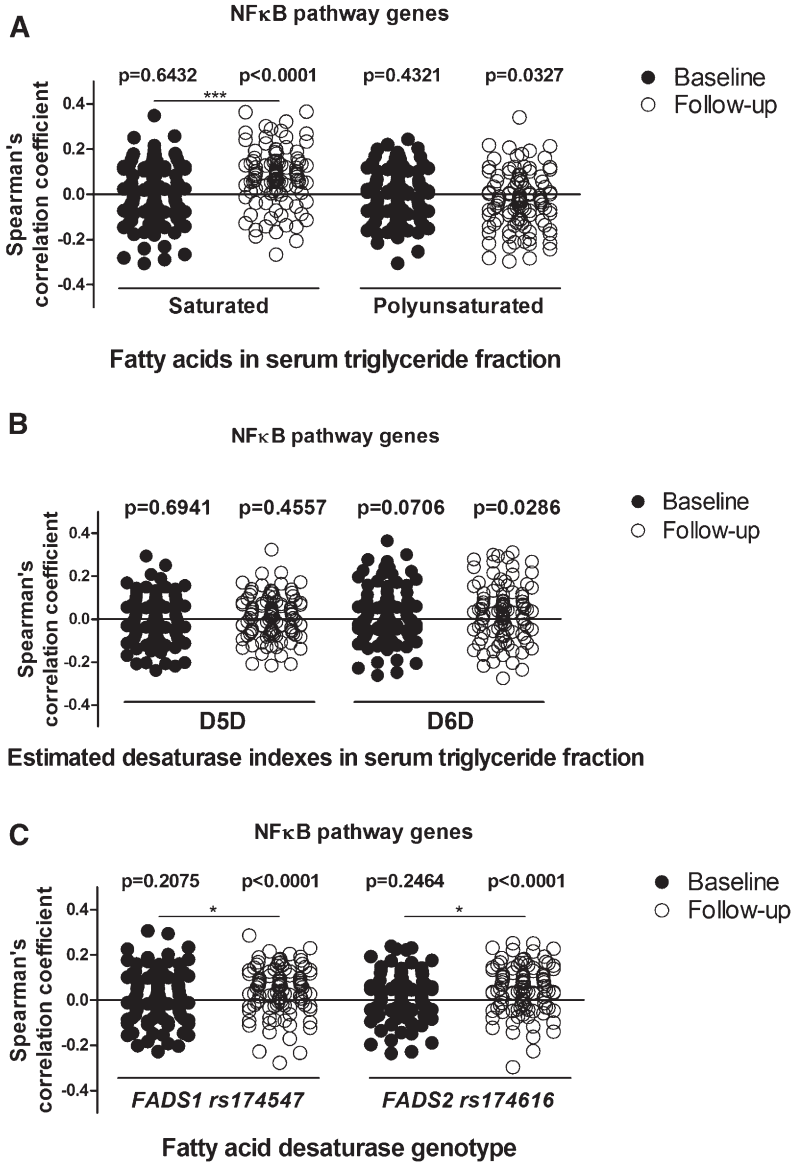


**Fig. 4.** Spearman's correlation coefficient values of TREx NFκB pathway gene expression in SAT with SFAs and PUFAs (A), estimated D5D and D6D enzyme activities (B), and genetic variations in the *FADS1* (rs174547) and *FADS2* (rs174616) genes (C) in the KOBS study. One sample *t*-test was used to analyze the deviation of correlation coefficients from 0 at baseline or follow-up. Paired *t*-test was used to compare correlation coefficients for genes at baseline and follow-up. $*P < 0.05$, $***P < 0.0001$.

In fact, there are several possible mechanisms that link serum FA composition to the regulation of *IL-1β* gene expression in AT. Serum FAs reflect AT FAs (21) and, thus, the link between serum FAs and *IL-1β* gene expression regulation in AT may, in fact, be showing the association between AT FAs and AT inflammation. Alternatively, it is known that FAs have hormone-like effects and are able to regulate gene expression (31) and, thus, serum FAs could also regulate gene expression in tissues. However, the exact mechanisms by which n-6 PUFAs and *FADS2* genotype coregulate AT *IL-1β* expression needs to be elucidated. We propose that the lack of significant correlations between *IL-1β* expression and FA composition in AT could be explained, in part, by the smaller sample size with AT samples. However, we could clearly demonstrate a correlation between serum and AT FA composition in the TG fraction (supplementary Table 5), demonstrating that serum samples, with some exceptions, can be used as markers of AT FAs. This is in line with previous studies demonstrating that plasma TGs are an important source of endogenous FA and are in constant interaction with AT FAs (32). We postulate that serum FA composition can be used as a marker of endogenous FA metabolism in AT in our study.

We could also verify the observation that the association of lower proportions of serum and AT PUFAs, D6D index, and *FADS1/2* genotypes on AT inflammation is revealed after weight loss using an NFκB-related gene expression panel. Because of limited sample size, our power was not high enough to identify individual genes related to serum and AT FA profile in the TG fraction when using false discovery rate-corrected *P* values (supplementary Table 3). However, as a group, we could verify that NFκB-related genes had, on average, a more positive correlation with serum SFAs and *FADS1/2* genotypes at follow-up when compared with baseline. Moreover, we could show, both in serum and in AT, a negative correlation between NFκB-related genes and PUFAs and a positive correlation between NFκB-related genes and estimated D6D enzyme activity at follow-up. Specifically, the finding that an association

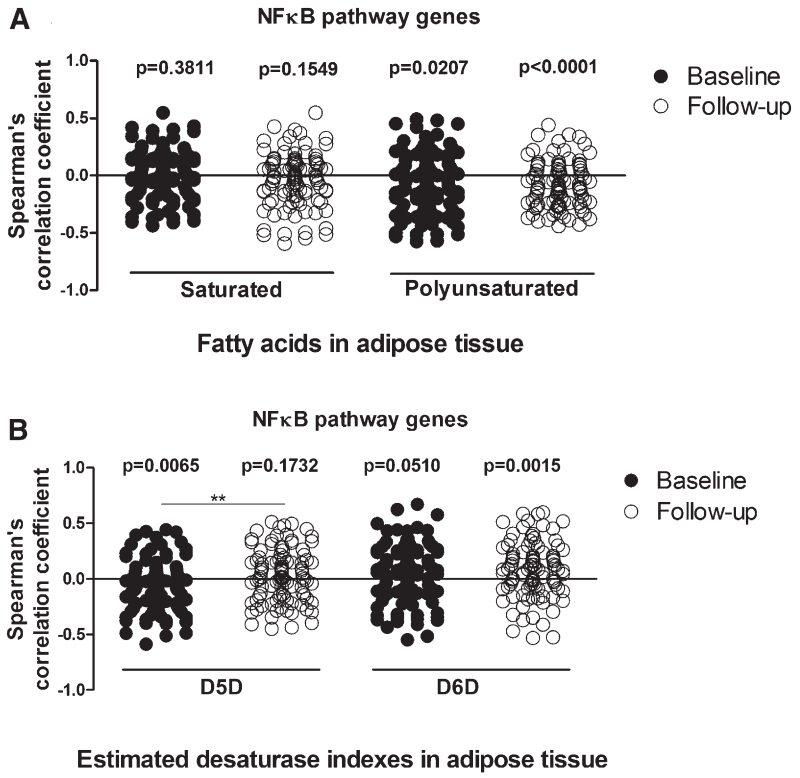


**Fig. 5.** Spearman's correlation coefficient values of TREx NFκB pathway gene expression in SAT with SFAs and PUFAs in AT (A) and estimated D5D and D6D enzyme activities (B) in the KOBS study. One sample *t*-test was used to analyze the deviation of correlation coefficients from 0 at baseline or follow-up. Paired *t*-test was used to compare correlation coefficients for genes at baseline and follow-up. $^{**}P < 0.01$.

between AT inflammation and *FADS1/2* genotypes was observed only after weight loss suggests an interaction between obesity (or obese state in general) and genotype (Fig. 4C). We suggest that the most likely explanation is that the effect of the genotype is masked by obesity-related AT inflammation and related comorbidities (e.g., hyperglycemia, hyperinsulinemia) at baseline. Furthermore, we suggest that the association is revealed after weight loss and concurrent improvement in IR. On the other hand, this implies that individuals with major alleles of the *FADS1* and *FADS2* genotypes with the higher estimated D6D enzyme activity may not benefit as much from weight loss with regard to AT inflammation (Fig. 3C, D).

There are some limitations in our study. First, the participants of the study were obese, even after weight reduction, and thus the results cannot be generalized to individuals with normal body weight. On the other hand, low-grade inflammation is closely related to obesity and, thus, it is difficult to investigate in lean individuals. We acknowledge the calculated enzyme activity indexes for FADSs are estimates of the actual enzyme activity. However, they have been widely used as surrogate markers for FA metabolism (33, 34). Finally, we clearly observed that for genetic association studies, our sample size was small because we did not observe the association of the *FADS1/2* SNPs with serum lipids (14, 17). However, we still had enough power to detect the direct effect on respective enzyme activities.

We conclude that the genetic variation in the *FADS1/2* genes associates with FA metabolism and AT inflammation, leading to an interaction between weight loss and *FADS1/2* genes in the regulation of AT inflammation. This supports the hypothesis that genetic regulation of endogenous FA metabolism interacts with obesity in the regulation of tissue inflammation.

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