**Effects of dietary fat on insulin secretion in subjects with the metabolic syndrome**

**Short title: Dietary fat and insulin secretion**

Hanne L Gulseth1,2,3\*, Ingrid MF Gjelstad1,2, Audrey Tiereny4,5,6, Danielle McCarthy7,8, Julie A Lovegrove7,9, Catherine Defoort10, Ellen E Blaak11, Jose Lopez-Miranda12, Aldona Dembinska-Kiec13, Ulf Risérus,14, Helen M Roche4, Christian A Drevon2 & Kåre I Birkeland1,15.

1Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital and Faculty of Medicine, University of Oslo, Oslo, Norway, 2Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Oslo, Norway, 3Department of Chronic Diseases and Ageing, Norwegian Institute of Public Health, Oslo, Norway, 4Nutrigenomics Research Group, UCD Conway Institute & UCD Institute of Food and Health, School of Public Health, Physiotherapy and Sports Science, University College Dublin, Dublin, Ireland; 5School of Allied Health, University of Limerick, Ireland; 6School of Allied Health, La Trobe University, Bundoora, Melbourne, Australia, 7the Hugh Sinclair Unit of Human Nutrition, Department of Food Biosciences, University of Reading, Reading, UK, 8Institute for Global Food Security, Northern Ireland Technology Centre, Queen’s University Belfast, Belfast, Nothern Ireland, 9Institute for Cardiovascular and Metabolic Research, University of Reading, Reading, UK, 10C2VN, INRA, INSERM, Aix Marseille Univ, Marseille, France, 11NUTRIM, School for Nutrition, Toxicology and Metabolism, Maastricht University Medical Centre, Maastricht, the Netherlands, 12Lipids and Atherosclerosis Research Unit, Instituto Maimonides de Investigacion Biomedica de Córdoba (IMIBIC)/Hospital Universitario Reina Sofia/Universidad de Cordoba and CIBER Fisiopatologia Obesidad y Nutricion (CIBEROBN), Instituto de Salud Carlos III. Spain, 13Department of Clinical Biochemistry, Jagiellonian University Medical College, Krakow, Poland, 14Department of Public Health and Caring Sciences/Clinical Nutrition and Metabolism, Uppsala University, Uppsala, Sweden, 15Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway.

\*Address correspondence and request for reprints to Dr Hanne L Gulseth, Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital, POB 4959 Nydalen, N-0424 Oslo, Norway. E-mail: h.l.gulseth@medisin.uio.no

**Keywords:** metabolic syndrome, diet, fat modification, insulin secretion, IVGTT, LIPGENE. MUFA, SFA

Supported by: LIPGENE, an EU Sixth Framework Program Integrated Project (FOOD-CT-2003-505944), the Norwegian Foundation for Health and Rehabilitation, South-Eastern Norway Regional Health Authority and Johan Throne Holst Foundation

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**Number of tables:** 3

**Number of figures:** 1

**Abstract word count**: 222

**Word count**: 2782**ABSTRACT**

**Objective:** Impaired insulin secretion and action contribute to the development of type 2 diabetes. Dietary fat modification may improve insulin sensitivity, whereas the effect on insulin secretion is unclear. We investigated the effect of dietary fat modification on insulin secretion in subjects with the metabolic syndrome.

**Design:** In a 12 weeks pan-European parallel, randomized controlled dietary intervention trial (LIPGENE) 486 subjects were assigned to four isoenergetic diets: high fat diets rich in saturated fat (HSFA) or monounsaturated fat (HMUFA); or low-fat, high complex carbohydrate diets with (LFHCC *n-*3) or without (LFHCC-control) 1.2 g/day of *n*-3 PUFA supplementation. Insulin secretion was estimated as acute insulin response to glucose (AIRg) and disposition index (DI), modeled from an intravenous glucose tolerance test.

**Results:** There were no overall effect of the dietary intervention on AIRg and DI in the total cohort, in neither the high fat nor LFHCC groups. We observed significant diet\*fasting glucose category interactions for AIRg (p=0.021) and DI (p=0.001) in the high-fat groups. In subjects with normal fasting glucose and preserved first phase insulin secretion, the HMUFA diet increased whereas the HSFA diet reduced AIRg (p=0.015) and DI (p=0.010).

**Conclusions**: The effects of dietary fat modification on insulin secretion were minor, and only evident in normoglycaemic subjects. In this case, the HMUFA diet improved AIRg and DI, as compared to the HSFA diet.

 **INTRODUCTION**

Subjects with the metabolic syndrome have insulin resistance and increased risk of insulin secretory dysfunction, and type 2 diabetes1, 2. Loss of insulin secretion is linked to the development of impaired glucose tolerance and type 2 diabetes3 andβ-cell dysfunction may predict who, among subjects with insulin resistance, eventually will develop impaired glucose tolerance or type 2 diabetes4-9. In the Diabetes Prevention Program, as well as in the Finnish Diabetes Prevention Study, lifestyle modifications with diet and exercise reduced the risk of developing type 2 diabetes10, 11, and also showed beneficial effects on the prevalence and features of the metabolic syndrome12, 13.

Dietary macronutrient composition may influence insulin sensitivity14-17, but little is known about the effect on insulin secretion18. Carbohydrate modification improved insulin secretion measured as the insulinogenic index in subjects with the metabolic syndrome19, whereas substituting dietary saturated fat (SFA) for monounsaturated fat (MUFA) in the KANWU study attenuated the reduction in insulin sensitivity, but did not affect insulin secretion in healthy subjects20. In contrast, a cross-sectional study from Spain suggested a favorable relationship of MUFA with insulin secretion based on fasting measurements of glucose and insulin (homeostasis model of insulin secretion (HOMAβ))21. In the long-term prospective CODAM cohort study, dietary MUFA intake was also associated with improved β-cell glucose sensitivity22 .The long-term effects of polyunsaturated fatty acids (PUFA) on insulin secretion in humans are sparsely investigated, although one study showed no beneficial effect of fish oil supplementation regardless of habitual dietary intake of *n*-3 and *n*-623. A more recent meta-analyses of feeding trials however showed that PUFA could have beneficial effects on insulin secretory capacity whether replacing carbohydrate, SFA, or MUFA24.

In the present study, we investigated the impact of an iso-energetic change in dietary fat quantity and quality on insulin secretion in a large European sample of subjects with the metabolic syndrome.

**SUBJECTS AND METHODS**

The LIPGENE study was a clinical 12 weeks parallel, randomized controlled dietary intervention trial, performed in eight European centers as part of the EU 6th Framework integrated project “Diet, genomics and the metabolic syndrome – LIPGENE” (<http://www.ucd.ie/lipgene/>). Randomization was completed centrally, stratified for age, sex and fasting plasma glucose concentration using the MINIM program (MINIM: allocation by minimization in clinical trials, Evans S, Royston P and Day S, Dept of Clinical Epidemiology, The London Hospital Medical College, UK) 25. The study was approved by local ethics committees at each of the eight intervention centers (Dublin, Reading, Oslo, Marseille, Maastricht, Cordoba, Krakow and Uppsala) and conformed to the Declaration of Helsinki. Written informed consent was obtained from all study participants. The study was registered with the US National Library of Medicine Clinical Trials registry (NCT00429195). The primary endpoint was to determine the effect of dietary fat modification on insulin sensitivity and these results have been reported previously 26. The effects of the intervention on insulin secretion were a pre-specified secondary endpoint.

**Subjects**

Study participants, Caucasian males and females aged 35-70 years with BMI 20-40 kg/m2, had the metabolic syndrome as defined by three or more of the following slightly modified NCEP ATP-III criteria 27; Levels of fasting plasma glucose > 5.5 mmol/L, triglycerides ≥1.5 mmol/L, HDL-cholesterol <1.0 mmol/L (males) or <1.3 mmol/L (females), systolic blood pressure (BP) ≥130 mmHg or diastolic BP ≥85 mmHg (or BP lowering medication), and waist circumference >102 cm (males) or >88 cm (females). Antihypertensive medication, hormone replacement therapy, multivitamin supplements and non-fatty acid based nutritional supplements were allowed if the subject adhered to the same regimen throughout the study. Exclusion criteria included pre-diagnosed diabetes, inflammatory diseases, use of statins and anti-inflammatory drugs, fatty acid supplements, alcohol abuse and a recent weight change ≥3 kg. A total of 486 subjects attended the pre-intervention investigational day, 417 participants completed the study and 404 subjects had a post-intervention intravenous glucose tolerance test (IVGTT) performed.

**Diets**

Subjects were randomly assigned to one of four isoenergetic diets differing in fat quantity or quality as previously described 28. In short, two high fat diets provided 38 % energy (E%) from fat: one with a high content (16 E%) of SFA (HSFA diet), and one with a high content (20 E%) of MUFA (HMUFA diet) and two low-fat, high-complex carbohydrate (LFHCC) diets contained 28 E% from fat, with diet LFHCC *n-*3 including 1.2 g/d supplement of very long chain marine *n-*3 polyunsaturated fatty acids (VLC *n*-3 PUFA) and diet LFHCC-control including a control high-oleic acid sunflower-seed oil capsule (Lipid Nutrition, Loders Croklaan, Wormerveer, The Netherlands). Dietary targets are presented in **Table 1**. A food exchange model was developed, and fat modified food products (margarines, cooking and baking fats, oils, dressings and biscuits) were supplied by Unilever (Vlaardingen, The Netherlands). All participants completed a three day weighed food record and a food frequency questionnaire (FFQ) to estimate dietary intake. Food records were also completed mid- and post-intervention to assess compliance. Nutrient composition was calculated using center- specific dietary analysis programs, including the nutrient composition of the LIPGENE intervention foods. To ensure adherence to the diets the dietitians/nutritionists regularly, at least every other week, contacted the participants for 24-hour diet recalls and completion of food use questionnaires. All diets were isoenergetic and investigators monitored subjects’ weight during the intervention period with the aim of maintaining weight.

**Clinical assessment**

Health status was screened by medical history and routine laboratory measurements. A questionnaire was used to monitor each subject’s level of physical activity, measured as total Baecke score 29, smoking habits, alcohol consumption and demographic data. Anthropometric measurements were recorded according to standard protocols. Weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively. Waist circumference was recorded at the midpoint between the lowest rib and the iliac crest to the nearest 0.1 cm. BMI was calculated as weight (kg)/height2(m2). BP was measured using an automatic BP measuring device following the European Society of Hypertension Guidelines 30. Measurement was performed with an appropriately sized cuff positioned at the heart level and the patient had been relaxed for minimum five minutes. At least two measurements were performed, and the average used for data processing.

**Insulin action and secretion**

An insulin-modified intravenous glucose tolerance test (IVGTT) was performed as described prior 31. After an overnight fast, a catheter was inserted into an antecubital vein for blood sampling and into a contralateral antecubital vein for glucose and insulin injection. At time 0 min, glucose (0.3 g/kg bodyweight) was given intravenously, and after 20 min a bolus of 0.03 IU/kg of human insulin (Actrapid, NovoNordisk, Denmark) was injected. Blood samples were drawn at -5, 0, 2, 4, 8, 19, 22, 30, 40, 50, 70, 90 and 180 min. Measures of insulin sensitivity (SI) were determined using the MINMOD Millennium Program (version 6.02, Richard N Bergman) 32. The acute insulin response to glucose (AIRg=first phase insulin response) was defined as the incremental area under the curve from time 0-8 minutes. Disposition index (DI) was calculated as the product of AIRg and SI, and was determined for the 330 subjects with complete pre and post SI modeling as carried out with the MINMOD programme.

**Biochemical analyses**

Plasma insulin concentrations were determined using a human immunometric assay kit (AutoDELFIA Insulin, Perkin Elmer Life Sciences, Wallac Oy, Turku, Finland) on a 1235 automatic immunoassay system (Wallac 1235 AutoDELFIA, Wallac Oy, Turku, Finland). Plasma total cholesterol, HDL-cholesterol, LDL-cholesterol, triacylglycerol (TAG) and glucose concentrations were determined with an ILAB 600 clinical chemical analyzer using enzymatic colorimetric kits (Instrumentation Laboratory, Warrington, UK). HDL-cholesterol and LDL-cholesterol were isolated using relevant kits (HDL-cholesterol kit, Instrument Laboratory, War

rington, UK and CH1350 Randox Laboratories Ltd, Antrim, UK).

**Statistical methods**

Baseline data are presented as group means and standard deviations (SD), and changes in biological parameters are given as mean per group and 95% CI. Differences between baseline parameters were investigated using ANOVA, non-parametric alternative Kruskal-Wallis and the chi-square test for categorical variables as appropriate. General linear models (two-way ANCOVA) were usedto assess differences in post-intervention parameter between groups, adjusted for baseline parameter and potential confounders (age, sex, center, and BMI) 33. Correlations between parameters were calculated with Spearman’s correlation coefficients. A two-sided P value <0.05 was regarded significant. Analyses were performed using SPSS® for Windows™ 24.0 (SPSS Inc, Chicago, Ill) and R 2.8.0 (R foundation for Statistical Computing, Vienna, Austria).

**RESULTS**

**Baseline characteristics of the study subjects**

Clinical characteristics of the 408 study participants (diet HSFA *n*=95, diet HMUFA *n*=109, diet LFHCC-control *n*=103 and diet LFHCC*n*-3 *n*=101) with available baseline IVGTT modeling are described in **table 2**; there were no differences between the dietary groups in baseline characteristics. The mean age was 54.4 ± 9.0 years, 45% were males, the mean BMI was 32.3 ± 4.1 kg/m2 and only 10 subjects had BMI < 25 kg/m2. Baseline parameters of insulin secretion (AIRg and DI) did not differ between completers and non-completers, and the non-completers were evenly distributed between dietary groups. In our cohort 33% were normoglycaemic, (fasting p-glucose < 5.6 mmol/L), 57 % had impaired fasting glucose (fasting p-glucose 5.6-7.0 mmol/L) and 10% had fasting glucose values ≥ 7.0 mmol/L. First phase insulin secretion at baseline declined across categories of fasting p-glucose, and there were significant differences between categories of fasting p-glucose for AIRg (P < 0.001) and DI (P < 0.001). SI did not differ (P=0.36)

**Effects on insulin secretion**

In the total cohort, there were no significant differences in the effects of the intervention on AIRg or DI between all dietary groups, nor between the two high fat dietary groups or the two LFHCC diets (all P > 0.05) (**Table 3**).

As insulin secretion differed across categories of fasting plasma glucose we tested whether insulin secretion differed between groups in normoglycaemic individuals. We demonstrated a significant interaction between diet group and fasting p-glucose category for the HMUFA versus the HSFA diet for both AIRg (P=0.021) and DI (P=0.001). In subjects with normal fasting p-glucose <5.6 mmol/l, insulin secretion was lower after the HSFA diet compared to the HMUFA diet, both measured as AIRg (P=0.015) and DI (P=0.010) (Figure 1). SI did not differ (P=0.74). In subjects with fasting p-glucose > 5,6 mmol/L the intervention did not influence insulin secretion. Excluding subjects with fasting plasma glucose ≥ 7.0 mmol/L did not alter these results. There was no interactions between diet\*category of fasting blood glucose for the LFHCC diets.

To explore these results further, we investigated the relationship between insulin secretion and dietary records in subjects with normal fasting glucose. High intake of SFA was associated with reduced insulin secretion, whereas high MUFA-intake enhanced insulin secretion. Change in DI correlated positively with the reported dietary MUFA E% after intervention (r=0.31, p=0.018), whereas both changes in AIRg and DI correlated inversely with reported total dietary SFA intake (r=-0.31, p=0.019 and r=-0.30, p=0.02, respectively). In addition the dietary intake of individual saturated fatty acids (12:0, 14:0, 16:0, 18:0) post-intervention correlated inversely with changes in both AIRg and DI (all p< 0.03).

**Dietary records and body weight**

Dietary composition at baseline was similar between diets, and dietary intakes at baseline and at the end of intervention are presented in **table 1**. Dietary compositional targets were achieved with aimed differences in fat quantity between the high-fat and the low-fat diets, and in fat quality between the HSFA and HMUFA diets28. Body weight remained unchanged in the two high fat dietary groups, but was slightly reduced on the two low fat diets (LFHCC-control: -0.9 (95%CI -1.3, -0.6) kg, and LFHCC *n-*3: -0.7 (95%CI -1.0, -0.3) kg)26.

**DISCUSSION**

In the present iso-energetic intervention study we found no major effects of intervention in dietary fat quantity and quality on insulin secretion in a population of subjects with the metabolic syndrome and high risk of developing type 2 diabetes. However, the metabolic syndrome is a very heterogeneous classification, and to this end we report interesting preliminary findings wherein the impact of a dietary intervention may depend, in part, on the baseline glycemic status of the cohort. The findings are in line with a small, randomized cross-over trial in 8 healthy young males, reporting no effect of dietary fatty acid saturation on IVGTT-estimated insulin secretion 34. Also in an epidemiological study among Swedish women there were no correlations between insulin secretory variables and habitual dietary fat intake 35. In the KANWU study, no effects on AIRg 20 or DI 23 were observed. The diets in the KANWU study were comparable to the HSFA and HMUFA diets in LIPGENE study and the study design was similar, although the mean plasma glucose was 5.2 mmol/L in KANWU and 6.0 mmol/L in the LIPGENE study.

First phase insulin secretion was significantly reduced in LIPGENE participants with elevated fasting glucose, as expected and previously reported 36, 37. Hence, it may be expected that only the group with normal fasting glucose and preserved first phase insulin secretion showed effect of the dietary intervention on insulin secretion. In this subgroup, there was a small, but significant detrimental effect of a SFA enriched diet as compared to a high MUFA diet. Thus, with regard to β-cell function, subjects with normal glucose levels may be more sensitive to dietary fat modification. Consistent with our results, Rojo-Martínez *et al* reported an association between MUFA and improved insulin secretion in a population-based study 21. Meal studies have also shown a beneficial effect on β-cell function of MUFA compared to SFA 38, 39. Acute stimulatory effects of fatty acids on glucose-stimulated insulin secretion have been demonstrated both in vitro and in vivo 18. In eight healthy volunteers the effect of dietary fatty acid quality was investigated and MUFA proved to have the greatest increase in glucose stimulated insulin secretion measured during hyperglycemic clamp 40. The mechanisms responsible for these differential effects of diets rich in SFA and MUFA are unclear. One possibility is an effect via the incretin system, as MUFA, but not SFA, has been shown to enhance GLP-1 secretion 38, 41. GLP-1 responses were not measured in the present study as insulin secretory response was studies after intravenous rather than after oral glucose exposure.

It has also been proposed that high daily intake of SFA in genetically prone subjects might lead to type 2 diabetes due to lipotoxicity. Accumulation of intramyocytic TAG may decrease insulin-stimulated glucose uptake and impair glucose oxidation, whereas large TAG stores in pancreatic β-cells may promote reduced insulin secretion18. Especially palmitic acid has been shown to be lipotoxic and induce ER stress42, 43.

We did not find an effect of marine omeaga-3 fatty acid supplementation of insulin secretion during low fat diets, which is in accordance with previous controlled studies34, 41. We cannot exclude the possibility that larger doses of n-3 PUFA supplementation than we used (1.2 g/day) or longer duration might have an effect.

The IVGTT is a sensitive tool44 to assess insulin secretion as loss of function usually is seen well before diabetes or even impaired glucose tolerance develops. However, the IVGTT may not be an optimal method for measuring insulin secretion in subjects with hyperglycemia as the first phase insulin secretion (AIRg) often vanishes above fasting p-glucose concentrations > 7.0 mmol/L 37. Pre-diagnosed diabetes mellitus was an exclusion criterion for the LIPGENE study, still 37 participants had a fasting p- glucose ≥ 7.0mmol/L when tested. We did not perform oral glucose tolerance tests; thus, most likely there are even more cases of undiagnosed type 2 diabetes subjects in our cohort. Another limitation of the IVGTT method as compared to the OGTT, is that it does not assess incretin-induced insulin secretion.

The LIPGENE study is one of the largest dietary intervention studies carried out in subjects with obesity and a dysmetabolic profile. With subjects from Northern, Southern, Eastern and Western Europe the dietary interventions were implemented in a heterogeneous dietary background. The dietary intakes, as assessed by weighed food records, were achieved with significant differences in fat quantity and quality between diets 28. However, lack of overall dietary effects due to poor adherence to the diets cannot be excluded. The study was designed to be isoenergetic and the subjects should not lose weight. Body weight did not change in the high fat dietary groups, but there was a small and statistically significant weight loss of < 1 kg in the low fat groups. The primary endpoint of the LIPGENE study was insulin sensitivity; therefore power calculations did not include estimates of insulin secretion, which limit the impact of the present analysis.

In conclusion the effects of dietary fat modification on β-cell function were minor in the total study cohort, but in normoglycaemic subjects the HMUFA diet was beneficial as compared to the HSFA diet. Further controlled studies should assess the possible effects of dietary fat modification on incretin-induced insulin secretion in subjects with a broad range of abnormalities in glucose metabolism.

**CONFLICT OF INTERESTS, FUNDING AND ACKNOWLEDGEMENTS**

The authors report no conflict of interests.

The study has been supported by LIPGENE - an EU 6th Framework Program Integrated Project (FOOD-CT-2003-505944); the Norwegian Foundation for Health and Rehabilitation; South-Eastern Norway Regional Health Authority; and the Norwegian Diabetic Association. Grants were also obtained from the Freia Chocolade Fabriks Medical Foundation, Direktør Johan Throne Holst Foundation for Nutrition Research, Norway.

We thank the LIPGENE participants and the staff at all the clinical centers for their enthusiasm and support: Trinity College Dublin/University College Dublin, Ireland; University of Reading, UK; Oslo University Hospital Aker/University of Oslo, Norway; INSERM, Marseille, France; Maastricht University, The Netherlands; Hospital Universitario Reina Sofía/University of Córdoba, Spain; Jagiellonian Medical College, Krakow, Poland and Uppsala University, Sweden.

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**Table 1**. Dietary intake at baseline and after 12 weeks of dietary intervention with aims for adjustments of fat intake in bold



HSFA: high fat diet rich in saturated fat; HMUFA: high fat diet rich in monounsaturated fat; LFHCC control:

low-fat, high-complex-carbohydrate diet with control supplement; LFHCC- n-3: low-fat, high-complex-carbohydrate

diet with very long-chain n-3 PUFA supplement; E%: energy %; EPA: eicosapentaenoic acid; DHA:

docosahexaenoic acid. Values represent means (SD). \*n = x, 71, 79, 79 and 76, respectively

**Table 2**. Means (SD) or percentages of baseline clinical characteristics across dietary groups

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **HSFA** | **HMUFA** | **LFHCC control** | **LFHCC n-3** |
| *n* | 95 |  | 109 |  | 103 |  | 101 |  |
| Age (years). | 54.2 | (8.6) | 54.9 | (8.7) | 54.1 | (9.3) | 54.8 | (9.9) |
| Sex (%males) | 53 |  | 45 |  | 48 |  | 47 |  |
| BMI (kg/m2) | 32.0 | (4.3) | 32.7 | (4.2) | 32.0 | (4.3) | 32.5 | (4.1) |
| Waist circumference | 105.9 | (9.7) | 106.8 | (11.2) | 107.0 | (11.2) | 106.4 | (9.9) |
| Body fat\* | 36.1 | (8.9) | 36.8 | (8.5) | 36.6 | (8.5) | 36.3 | (9.0) |
| F-plasma glucose (mmol/L)  | 6.0 | (0.8) | 5.9 | (0.7) | 6.0 | (0.8) | 5.9 | (0.9) |
| F-triacylglycerol (mmol/L) | 2.0 | (1.1) | 1.8 | (0.8) | 1.8 | (0.9) | 1.8 | (0.9) |
| HDL-cholesterol | 1.04 | (0.24) | 1.09 | (0.29) | 1.09 | (0.28) | 1.11 | (0.29) |
| LDL-cholesterol | 3.2 | (1.0) | 3.1 | (1.0) | 3.2 | (1.0) | 3.4 | (1.1) |
| Total cholesterol | 5.3 | (0.8) | 5.3 | (0.9) | 5.3 | (1.0) | 5.4 | (0.9) |
| Smoke (% yes) | 35 |  | 28 |  | 29 |  | 37 |  |
| Physical activity (AU)† | 7.6 | (1.2) | 7.7 | (1.3) | 7.7 | (1.1) | 7.7 | (1.2) |

HSFA: high fat diet rich in saturated fat; HMUFA: high fat diet rich in monounsaturated fat;

LFHCC control: low-fat, high-complex-carbohydrate diet with control supplement; LFHCC- n-3: low-fat, high-complex-carbohydrate diet with very long-chain n-3 PUFA supplement; BMI: body mass index; AU: arbitrary unit of physical activity. Values represent means (SD). There were no significant differences between dietary groups at baseline in an ANOVA test.

\* n = 91, 105, 101 and 96, respectively

† n = 66, 78, 74 and 77, respectively

**Table 3.** Adjusted means and 95% CI for acute insulin response to glucose (AIRg) and disposition index (DI) at baseline and end of intervention.



All values represent adjusted means (age, sex, center and BMI) and 95% confidence intervals. Analysis of covariance model with adjustment for baseline value of the respective values including age, sex, center, and BMI. HSFA: High fat diet rich in saturated fat; HMUFA: High fat diet rich in monounsaturated fat; LFHCC control: Low-fat, high-complex-carbohydrate diet with control supplement LFHCC-n-3: Low-fat, high-complex-carbohydrate diet with very long-chain n-3 PUFA supplement. AIRg: acute insulin response to glucose, DI: disposition index

\* n = 96, 109, 103 and 96 respectively

† n = 74, 94, 84 and 78 respectively

**Figure legends**

Figure 1. Means (95% CI) disposition index (DI) after intervention, adjusted for baseline DI, age, sex, center, and BMI in the high fat diet rich in saturated fat (n=35), and the high fat diet rich in monounsaturated fat (n=26). P–value: Analysis of covariance model with adjustment for baseline value of the respective value, age, sex, center and BMI