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Samocha-Bonet, D.; Campbell, L. V.; Viardot, A.; Freund, J.; Tam, C. S.; Greenfield, J. R. & Heilbronn, Leonie Kaye (2010), 'A family history of type 2 diabetes increases risk factors associated with overfeeding', Diabetologia 53(8):1700-1708.*)*

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http://hdl.handle.net/2440/61472

A family history of type 2 diabetes increases risk factors associated with overfeeding

Samocha-Bonet D[1], Campbell LV[1], Viardot A[1], Freund J[2], Tam CS[1,3], Greenfield JR[1], Heilbronn LK[1,4]

[1]Diabetes & Obesity Program, Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, Australia

[2]Department of Nuclear Medicine, St Vincent's Hospital, 390 Victoria Street, Darlinghurst, NSW 2010, Australia

[3]Institute of Diabetes and Endocrinology, The Children's Hospital at Westmead, 212 Hawkesbury Rd, NSW 2145, Australia

[4]CCRE, Nutritional Physiology, Department of Medicine, The University of Adelaide

Short Title: Metabolic Health after Overfeeding in Humans

Corresponding author:

Leonie Heilbronn, CCRE Nutritional Physiology, Department of Medicine, University of Adelaide, Frome Rd, SA, 5005, Australia.

Phone: +6182224900

Fax: +6182233870

leonie.heilbronn@adelaide.edu.au

Word count: abstract: 246; Text: 3531

Abstract

Aims: To test prospectively whether healthy individuals with a family history of type 2 diabetes are more susceptible to adverse metabolic effects during experimental overfeeding.

Methods: We studied the effects of 3- and 28-days of overfeeding by 1250 kcal/day in 41 sedentary individuals with and without a family history of type 2 diabetes (FH+ and FH-). Measures included weight, fat distribution (CT) and insulin sensitivity (hyperinsulinemic-euglycemic clamp).

Results: Body weight was increased at +3 and +28-days in both groups (p<0.001), with FH+ gaining significantly more weight at +28-days (3.4 ± 1.6 vs. 2.2 ± 1.4 kg, p=0.02). Fasting serum insulin and C-peptide were increased at +3 and +28-days in both groups, with greater increases in FH+ for insulin at +3 and +28-days (p<0.01) and C-peptide at +28-days (p<0.05). Fasting glucose also increased at both time points, but without significant group effect (p=0.1). Peripheral insulin sensitivity decreased in the whole cohort at +28-days (54.8±17.7 to 50.3±15.6 μmol min^{-1} kgFFM^{-1}, p=0.03), and insulin sensitivity by HOMA-IR decreased at both time points (p<0.001) and to a greater extent in FH+ (p=0.008). Liver fat, subcutaneous and visceral fat increased similarly in both groups (p<0.001).

Conclusions: Overfeeding induced weight and fat gain, insulin resistance and hepatic fat deposition in healthy individuals. However, individuals with a family history of type 2 diabetes gained more weight and greater insulin resistance by HOMA-IR. This study suggests that healthy individuals with a family history of type 2 diabetes are predisposed to adverse effects of overfeeding.

Trial registry number: NCT00562393 (www.clinicaltrials.gov).

Key words: First-degree relatives of type 2 diabetes individuals; Insulin resistance, Overfeeding, Liver fat

Abbreviations

CT, computed tomography

DSAT, deep subcutaneous adipose tissue

DXA, dual energy X-ray absorptiometry

FCS, fat cell size

FH, family history

FFM, fat free mass

FM, fat mass

GIR, glucose infusion rate

HU, Hounsfield units

39 MUFA, monounsaturated fatty acid

40 MRS, magnetic resonance spectroscopy

41 NEAT, non-exercise activity thermogenesis

42 PYY, peptide YY

43 PUFA, polyunsaturated fatty acid

44 SAT, subcutaneous adipose tissue

45 SF, saturated fatty acid

46 SSAT, superficial subcutaneous adipose tissue

47 VAT, visceral adipose tissue

48

Introduction

Over-nutrition and sedentary lifestyle are major causes of the obesity epidemic, which is associated with increased risk of related metabolic disorders including hypertension, coronary artery disease, insulin resistance and type 2 diabetes. First-degree relatives of individuals with a family history of type 2 diabetes (FH+) are at increased risk of developing type 2 diabetes [1, 2]. The mechanisms leading to this are not entirely clear, although defects already identified in this population include a greater tendency towards insulin resistance [2, 3], pancreatic beta cell impairment [4, 5], central adiposity [6], increased inflammation [7], increased intramyocellular lipid [8] and reduced mitochondrial function [8, 9]. We and others have also reported that these individuals have an impaired ability to respond to dietary challenge, including impaired fatty acid oxidation in response to a single high fat meal [10] or 3-days isocaloric high fat feeding [11].

Short term experimental overfeeding is a model often used in animal studies to induce insulin resistance and associated metabolic defects. In humans, this model was previously applied in lean healthy individuals. The observed outcomes from these studies include increases in fasting insulin and glucose [12], increases in energy expenditure [13] and intramyocellular triacylglycerol content [14] and decreases in peripheral [15] and hepatic insulin sensitivity [12]. The aim of the present study was to examine prospectively the effects of 3 and 28-days of overfeeding on body weight, fat distribution and insulin sensitivity and the relationships between these factors in healthy individuals with and without a family history of type 2 diabetes. We hypothesised that overfeeding will induce a greater adverse effect in FH+ individuals.

Methods

Subjects Sedentary, non-smoking, non-diabetic men and women who either reported no family history of type 2 diabetes (FH-) or at least one first-degree relative with type 2 diabetes (FH+) were recruited by advertisements in local newspapers. Subjects were excluded if weight had changed by > 2 kg in the preceding 6-months, if they exercised more than 60 min per week, if they were taking medications known to affect insulin sensitivity or blood pressure or if they had a personal history of type 2 diabetes or cardiovascular disease. Forty one individuals were recruited; one male FH- subject did not complete the study due to a viral infection. The study protocol was approved by the Human Research and Ethics Committee at St Vincent's Hospital, Sydney. Subjects provided informed written consent before commencement. The study was registered as a Clinical Trial at www.clinicaltrials.gov, registration# NCT00562393.

Diets Estimated energy requirements were calculated for each subject based on fat-free mass (FFM) and fat mass (FM) using equations previously generated by doubly-labelled water and intake-balance techniques [16-18]. A trained dietician then planned individual menus for participants. Study timeline and food consumption regimen are outlined in Fig 1. Briefly, complete 3-day food intakes were supplied to each participant before each metabolic study such that from day -3 to day 0 all foods were provided at baseline energy requirements with a nutrient composition of 30% fat, 15% protein and 55% carbohydrates. On days 0 - 3 and 25 - 28 all foods were provided at baseline energy requirements plus 1250 kcal/day with a nutrient composition of 45% fat, 15% protein and 40% carbohydrates. During the overfeeding phase we aimed to double the amount of fat intake by providing 3 high energy-high fat snacks per day, each providing ~250 kcal (e.g. potato crisps, chocolate bars, cheesecake) and a liquid oil-based supplement (Benecalorie®, Novartis, Basel, Switzerland, 330 kcal) mixed in a dairy dessert (~200 kcal). On days 3 – 25 of overfeeding, subjects were instructed to consume their regular diets and were provided with the above snacks and supplement to achieve an intake of 1250 kcal/day above baseline energy requirements. They were required to fill out checklists daily reporting which snacks were consumed, complete 3-day diet diaries once before study commencement and twice during the overfeeding phase and to meet the study dietician weekly. The checklists were reviewed during weekly weigh-ins by the study dietician so that any deviations from protocol were quickly identified and alternative options could be provided, to improve adherence to the diet plan. Diets were analysed for macronutrients and fatty acid composition using FoodWorks 2007 based on the Australian foods database (Xyris Software). Thirty two subjects returned the diet diaries of both study phases (Table 1).

Metabolic Testing Subjects attended the clinical research facility at 8am after a 12-hour overnight fast at baseline, +3 and +28-day of overfeeding (Fig 1). Baseline and +28-day visits were identical; weight, height, blood pressure were measured in a hospital gown after voiding and fasting blood samples were drawn. After 30 min supine rest, resting metabolic rate (RMR) and RQ were determined for 30 min (ParvoMedics Inc. UT, US). A peri-umbilical subcutaneous fat biopsy was performed as described previously [19]. Insulin sensitivity was then measured by a 2-hour hyperinsulinemic-euglycemic clamp (60mU m^{-2} min^{-1}), as previously described [10]. Glucose was infused at a variable infusion rate to maintain glucose at 5.0 mmol/l and the steady state glucose infusion rate (GIR) was calculated between 90 and 120 min. Indirect calorimetry was repeated in conjunction with the steady state measurement. Stanford 7-day activity recall tests were also administered at baseline and after 28-days of

overfeeding [20]. At +3-days of overfeeding, weight and blood pressure were measured, fasting blood samples were obtained and indirect calorimetry performed. Subjects attended the Clinical Research Facility weekly for weight follow-up, snack collection and consultation with the study dietician.

Body Composition Fat mass, fat-free mass and central abdominal fat were assessed at baseline and +28-day of overfeeding by dual energy X-ray absorptiometry (DXA, Lunar DPX-Lunar Radiation, Madison WI), as previously described [21]. Three cross-sectional computed tomography (CT) scans (Phillips Gemini GXL), 1cm-width, centred on the L2-L3 and L4-L5 disc space, and the T12-L1 disc space were also performed to assess abdominal adipose tissue distribution and hepatic fat content. Abdominal areas of adipose tissue were defined by attenuation values of -50 to 150 Hounsfield units (HU), as previously described [19]. CT images were analysed using Gemini (GXL Host System). Two subjects did not undergo CT scans. L4-L5 superficial and deep subcutaneous adipose tissue were not analysed in 4 subjects and the spleen was not visualised in 2 subjects.

Fat Cell Size Measure Subcutaneous adipose biopsies were fixed in Bouin's, dehydrated, paraffin embedded and then sectioned (4μm thick). Sections were stained with haematoxylin and eosin. Digital images were captured using a camera (triCCD; Sony, Paris, France) and diameters measured using Perfect Image software (Claravision, Orsay, France). Fat cell size (FCS) was measured in 31 subjects who had histological samples available pre- and post-intervention. Adipocyte diameter measurement was performed blindly and for at least 2 fields of view. The mean diameter was calculated from an average of 400 cells per sample.

Biochemical Analysis Glucose was analysed using a glucose oxidase electrode (YSI Life Sciences). Fasting serum insulin, C-peptide and leptin were assayed by radioimmunoassay (Linco Research, St Charles, USA). HOMA-IR was calculated as [fasting insulin (mU/l)*fasting glucose (mmol/l)]/22.5. HDL cholesterol and triacylglycerol were evaluated by enzymatic colorimetry (Roche, Indiana, USA). LDL was calculated by the Friedewald equation. NEFA were measured by enzymatic colorimetry assay (Wako, Osaka, Japan).

Statistical Analysis Data is presented as mean ± SD unless otherwise stated. Statistics were analysed with SPSS 15 (Chicago, IL). Leptin and insulin data were not normally distributed and log transformed for analysis. Baseline differences between groups were analysed by one-way ANOVA. All other data was analysed using repeated measures with respect to group and time, and an intention to treat approach without carrying forward data on the one dropout. Bonferroni post-hoc was performed and further analysis was performed by independent t-test. Linear regression at baseline (n = 41) was used to generate equations for predicting RMR with FFM and FM in the model as previously described [16]. Correlations were performed using Pearson's correlation coefficient. Significance was set at $p < 0.05$.

160   **Results**

161

162   *Baseline Characteristics*

163   Baseline characteristics by group are shown in Table 2. There were no detectable differences
164   between groups at baseline with respect to age, weight, BMI, blood pressure, fasting glucose,
165   insulin, C-peptide, leptin, lipid profile or peripheral insulin sensitivity. The only difference
166   was reduced carbohydrate oxidation in response to insulin infusion during the
167   hyperinsulinemic-euglycemic clamp (ΔRQ) in FH+. At baseline, peripheral insulin sensitivity
168   was related to liver/spleen ratio (r = 0.5, p = 0.001) and visceral adipose tissue (r = -0.4, p =
169   0.01). Fat cell size correlated with percent body fat by DXA (r = 0.4, p = 0.04), subcutaneous
170   adipose tissue (r = 0.4, p = 0.02), serum NEFA (r = 0.4, p = 0.02), triacylglycerol (r = 0.5, p =
171   0.006) and insulin resistance by HOMA-IR (r = 0.5, p = 0.007).

172   *Diet Diary and Physical Activity Questionnaire Analysis*

173   Reported dietary intakes at baseline and during overfeeding by group are given in Table 1.
174   Dietary fat (g) approximately doubled in the overfeeding phase. Carbohydrate and protein
175   intake also increased (p <0.0001). There were no significant differences in energy,
176   carbohydrate or protein intake between groups, although a tendency was noted for FH+ to
177   consume more total energy (p = 0.15) and more fat during overfeeding (p = 0.07). The
178   average self-reported consumption of snacks during the overfeeding period was 92±14 and
179   95±9% in FH- and FH+, respectively (p = 0.4). Reported levels of physical activity were
180   similar at baseline (230±8 and 232±12 METs-hr/week, in FH- and FH+, respectively, p = 0.7)
181   and did not change with overfeeding (229±7 and 231±7 METs-hr/week, in FH- and FH+
182   respectively, time p = 0.4, group p = 0.8).

183   *Weight and Fat Distribution Changes in Response to Overfeeding*

184   As expected, overfeeding resulted in significant weight gain at +3 and +28-days (Fig 1A). At
185   3-days, weight gain was not different between groups. At 28 days, FH+ individuals had
186   gained 1.2 kg more than FH- individuals (p = 0.03). Weight gained as percentage of baseline
187   weight was 0.7±0.9 and 1.0±0.7% in FH- and FH+ at +3-days, respectively (p = 0.4) and
188   3.1±2.0 and 4.4±2.0% in FH- and FH+ at +28-days, respectively (p = 0.05). Fat mass, fat-free
189   mass, central fat by DXA and visceral and subcutaneous adipose tissue volume by CT
190   increased similarly in both groups (Table 3). Circulating leptin increased significantly with
191   weight gain and to a greater extent in FH+ at +28-days (Fig 1B), consistent with greater
192   weight gain in this group. The increase in circulating leptin at +3- and +28-days correlated
193   with weight gain at these time points (r = 0.3, p = 0.03 and r = 0.5, p = 0.003, at +3- and +28-
194   days, respectively). A preferential fat gain in the L2-L3 visceral depot was observed in the
195   whole cohort increasing from 33±15% at baseline to 34±14% at +28-days of overfeeding (p =
196   0.008). However, this was abolished when baseline visceral volume was included as a
197   covariate (p = 0.1). Liver fat increased significantly in response to overfeeding similarly in
198   both groups (Table 3) and correlated with weight gain (R^2 = 0.17, p = 0.01). Abdominal fat
199   cell size did not change (Table 3).

200   *Metabolic Responses to Overfeeding*

201   Fasting glucose increased significantly at +3 and +28-days of overfeeding (p <0.01), but this
202   response was not statistically different between groups (Fig 1C, p = 0.1). Fasting insulin and
203   C-peptide increased at +3 and +28-days of overfeeding (Fig 1D and 1E), with a greater

increase in insulin in FH+ individuals at both time points (p <0.01, Fig 1D) and in C-peptide at +28-days (p <0.05, Fig 1E). Accordingly, HOMA-IR (reflecting fasting insulin resistance) increased significantly in both groups (p <0.005 for both time points; Fig 1F), with the increase more pronounced in FH+ individuals (p = 0.003, Fig 1F). Peripheral insulin sensitivity measured by the hyperinsulinemic-euglycemic clamp at +28-days decreased significantly in the whole cohort from 54.8±17.7 to 50.3±15.6 µmol min^{-1} kgFFM^{-1} at +28-days of overfeeding (p = 0.03), but was not different between groups. Total and HDL-cholesterol were significantly increased in response to overfeeding similarly in both groups (4.6±1.0 to 4.8±1.0, p = 0.01 for cholesterol and 1.3±0.3 to 1.4±0.4, p <0.0001 for HDL). Blood pressure, LDL-cholesterol and triacylglycerol levels were unchanged (data not shown). Fasting NEFA levels were significantly suppressed at +3-days (p <0.001) and returned to basal at +28-days overfeeding, without group effect (p = 0.4, Fig 1G). Plasma NEFA were suppressed at the hyperinsulinemic state (by clamp) at both baseline and +28-days of overfeeding (p <0.0001), without group difference at both time points (data not shown). Similarly, fasting RQ increased at +3-days (p <0.0001) and returned to basal at +28-days, without group effect (p = 0.8, Fig 1H). The ΔRQ during the clamp was not altered by overfeeding (data not shown). Baseline absolute RMR was not different between groups (Table 2) and increased at +3-days in both FH- and FH+ (1315±260 to 1370+265 kcal/d and 1440±160 to 1530±195 respectively, p = 0.01) and at +28-days (1375±250 and 1525±220 kcal/day in FH- and FH+ respectively, p = 0.01). Baseline RMR adjusted for FFM was not different between groups or in response to overfeeding (data not shown). We also calculated predicted RMR at 28-days based on equations derived at baseline (RMR = 158.8+19.91*FFM+10.37*FM), as previously described [16] and found no difference between the predicted and measured RMR at +28-days overfeeding (data not shown). We did not perform DXA measures at day 3 but repeated this analysis using body weight at baseline (RMR = 344.9+14.0*Weight). Similarly, we found no difference at 28-days. However, at day 3, measured RMR (1454±40 kcal/day) was significantly elevated above weight-predicted RMR (1384±26 kcal/day). There was no difference between groups in this response (data not shown). The increase in thermogenesis in response to insulin infusion was 114 ± 164 kcal/day at baseline and 125 ± 148 kcal/day at overfeeding, both were not different between groups (data not shown).

Discussion

High fat overfeeding induces insulin resistance in rodent models in as little as 3-weeks [22, 23]. In this study, we established that 28-days of overfeeding induced weight gain and peripheral insulin resistance in healthy non-diabetic individuals. We also showed that fat was deposited in the liver and we established that individuals who report a family history of type 2 diabetes gained more weight and developed greater insulin resistance by HOMA-IR when provided with identical dietary instructions.

This is the first study to compare the effects of experimental overfeeding in individuals with and without a family history of type 2 diabetes and we observed that weight gain was higher in FH+ individuals. Moreover, since overconsumption of 10.5 kcal is required to gain 1 g of body weight [24, 25] and assuming no compensatory change in energy expenditure, we predicted a maximum weight gain of 3.3 kg with 28-days of overfeeding. We observed that FH+ individuals gained approximately the predicted weight whereas FH- individuals gained less than predicted. It should be emphasised that participants were free-living and self selecting their foods for most of the study. Therefore, this difference may be explained by dietary compliance and may suggest that FH- individuals are less able to over eat. Consistent with this, a trend towards greater fat and energy consumption was observed in FH+ individuals. This outcome is of great interest as identical dietary instruction and food options were provided to both groups. Although FH+ gained on average 1.2 kg more at day 28, we did not detect significant group differences in the compartmental gain by CT and DXA. This apparent inconsistency may stem from the lower reproducibility and higher variability of DXA [26] and CT estimates as compared to a single scale weight. Interestingly, we have previously reported impaired peptide YY (PYY) secretion in response to a meal in FH+ individuals [27], which may contribute to reduced satiety and facilitate weight gain. Even the classical experimental overfeeding studies [28-30] in which participants were incarcerated and all foods were provided, have shown wide variability in weight gain in response to controlled overfeeding. Furthermore, the experimental weight gain study in twins demonstrated a heritable component to weight gain with a much greater variability in weight gain between, than within, twin pairs [30]. In those studies, since energy intake was very carefully controlled, it is likely that differences in weight gain were due to variations in thermogenic response to overfeeding and possibly non-exercise activity thermogenesis (NEAT) [31]. In this study, we can establish that the weight gain differences between groups were not due to differences in compensatory changes in resting energy metabolism or physical activity levels by questionnaire, since more complex measures of activity were not used.

Overfeeding increased fasting insulin, glucose and peripheral insulin resistance in the whole cohort. This is consistent with previous studies of short-term (3-days) [15] and long-term (4.5-months) overfeeding [32] in healthy lean men. Our initial hypothesis was that FH+ would have greater metabolic defects associated with overfeeding. Indeed, we observed that fasting serum insulin and C-peptide increased more in individuals with a family history of type 2 diabetes. Fasting blood glucose tended to increase more in FH+ individuals at +3-days of overfeeding and may account for the significant increase in insulin in this group. Notably, the insulin increase was maximal after just 3 days of overfeeding, which was during a time when all foods were provided to participants and prior to any detectable weight gain differences between groups. Consistently, in response to 5-days overfeeding, healthy lean men increased insulin secretion during IVGTT [12]. In longer term overfeeding studies, reduced insulin clearance was observed after 4.5-months and significant weight gain in lean

young men [32]. These findings are similar to those in moderately fat fed dogs, where an initial increase in beta cell secretion is observed at 6-weeks, which is no longer evident at 3-months when hyperinsulinemia is maintained via reduced insulin clearance by the liver [33].

There is increasing evidence to suggest that the location of fat deposition may be more important than the total amount of fat stored in obese individuals [19]. For example, ectopic deposition of lipid within the liver is closely associated with traits of the metabolic syndrome [19, 34]. Consistent with this, we also observed a relationship between liver fat by CT and peripheral insulin resistance in this non-diabetic cohort at baseline. Gold-standard measures of liver fat were not conducted in the present study however, findings by magnetic resonance spectroscopy (MRS) and CT measurement of liver fat content are closely correlated [35]. Interestingly, we observed that deposition of fat within the liver was increased in response to 28-days of overfeeding and although we did not detect a difference between groups, the increase in fat was closely aligned with the amount of weight gained. Deposition of fat in the liver in response to overfeeding has previously been shown in high fat fed rodents and dogs [36, 37] and in response to 3-day high fat-high energy diet in healthy lean men by MRS [38]. Interestingly, moderate calorie restriction for 2-days decreases liver fat in the obese [39]. Increased visceral fat is also closely associated with insulin resistance [40] and increased visceral adipose tissue is observed in BMI-matched insulin resistant FH+ individuals [6]. In this study, visceral adiposity was similar between groups at baseline and FH+ individuals were not more likely to deposit fat in the visceral compartment in response to overfeeding. Increased fat cell size is also observed in insulin resistance and may represent the failure of the adipose tissue mass to expand to accommodate increased energy intakes [19]. We did not detect an increase in average FCS with the moderate weight gain achieved in this study. This result is in contrast to an historical experimental overfeeding study [28], but that intervention was longer with much higher weight gain.

There is some evidence to suggest that post-obese individuals do not appropriately oxidise dietary fat, which may predispose them to weight regain [41]. There is also marked variability in the capacity to switch appropriately between fat and carbohydrate oxidation between individuals [42]. This response has been termed metabolic flexibility [43] and has been associated with insulin resistance [11] and may also predispose to weight gain. In this study, we observed impaired metabolic flexibility in response to insulin infusion in FH+ at baseline. However, this defect was not altered by overfeeding and there was no difference between groups in the fasting rates of fatty acid oxidation at baseline or during overfeeding and thus is unlikely to have contributed to increased weight gain observed in FH+. Overfeeding initially suppressed fatty acid oxidation. This may be due to suppressed lipolysis of adipose tissue as evidenced by reduced plasma NEFA and mediated by the increase in insulin. Interestingly, fasting levels of fat oxidation and plasma NEFA returned to basal by 28-days despite continuation of overfeeding, possibly as peripheral insulin resistance increased.

In conclusion, short-term overfeeding induced insulin resistance and deposition of fat in the liver in healthy men and women. Individuals with a family history of type 2 diabetes were more susceptible to weight gain and developed greater insulin resistance by HOMA-IR which was evident even prior to any detectable difference in weight gain. This study suggests that healthy individuals with a family history of type 2 diabetes are predisposed to adverse effects of overfeeding, which may help explain their susceptibility to develop type 2 diabetes in an obesogenic environment.

Acknowledgements

338   **Table 1** Diet diaries analysis at baseline and during overfeeding by group

	FH-		FH+		*p*	
	Baseline	**Overfeeding**	**Baseline**	**Overfeeding**	**Time**	**Group**
Energy (kcal)	1900±590	2890±640	2080±630	3360±910	0.0001	0.15
Fat (g)	76±28	148±35	79±33	167±36	0.0001	0.07
PUFA (g)	13±7	17±5	12±7	18±5	0.001	0.4
MUFA (g)	28±10	71±19	28±12	80±14	0.0001	0.1
SF (g)	28±12	50±15	31±16	59±18	0.0001	0.2
Carbohydrate (g)	202±58	258±62	225±66	321±130	0.0001	0.2
Protein (g)	84±32	113±36	99±38	131±30	0.0001	0.8
Alcohol (g)	5±9	5±8	7±9	5±7	0.3	0.4

339   Data based on n = 32 (19 FH- and 13 FH+)

340   PUFA- polyunsaturated fatty acid, MUFA- monounsaturated fatty acid, SF- saturated fatty
341   acid

342

343   **Table 2** Baseline characteristics of study participants

	Whole cohort	FH-	FH+	*p*
M/F	21/20	12/12	9/8	
Age	37±12	37±12	38±12	0.7
Weight (kg)	75.0±12.0	73.5±13.0	77.3±10.0	0.3
BMI (kg m^{-2})	25.6±3.5	25.1±3.1	26.4±4.1	0.3
Systolic BP (mmHg)	113±12	110±13	117±10	0.08
Diastolic BP (mmHg)	72±9	72±10	73±7	0.6
Glucose (mmol/l)	4.5±0.4	4.5±0.4	4.5±0.2	1
Insulin (pmol/l)	69.5±23.7	70.2±27.5	68.6±17.6	0.8
C-peptide (pmol/l)	496±13	486±179	510±145	0.6
Leptin (µg/l)	13.5±9.9	14.0±10.7	12.7±9.0	0.7
GIR (µmol min^{-1} kgFFM^{-1})	54.0±18.2	56.4±19.7	50.7±15.7	0.3
HDL (mmol/l)	1.3±0.3	1.3±0.4	1.2±0.3	0.2
LDL (mmol/l)	2.8±0.9	2.7±0.9	2.9±0.9	0.3
Cholesterol (mmol/l)	4.6±1.0	4.5±1.1	4.7±1.0	0.5
Triacylglycerol (mmol/l)	1.1±0.4	1.1±0.4	1.2±0.5	0.5
RMR (Kcal/day)	1388±222	1344±28	1443±30	0.2
RQ basal	0.81±0.04	0.80±0.04	0.81±0.03	0.7
ΔRQ Clamp	0.10±0.04	0.11±0.05	0.09±0.03	0.04

344   GIR- glucose infusion rate, FFM- fat free mass

345

346 **Table 3** Body weight, percent body fat and central fat (by DXA), abdominal fat distribution
347 and liver density (by CT) and fat cell size at baseline and post 28-days of overfeeding in
348 subjects with or without a family history of type 2 diabetes

	FH-		FH+		*p*	
	Baseline	**Overfeeding**	**Baseline**	**Overfeeding**	**Time**	**Group**
Weight (Kg)	73.9±13.1	76.1±13.3	77.3±10.0	80.7±10.4	0.0001	0.02
Fat mass (%)	33±9	34±8	34±7	35±7	0.0001	0.9
Fat-free mass (Kg)	48.1±9.3	48.6±9.5	49.8±7.6	50.8±7.5	0.0001	0.2
Central fat (kg)*	1.8±0.8	1.9±0.7	2.2±0.8	2.4±0.7	0.0001	0.2
L2/L3 VAT (cm^2)	78±74	92±75	105±76	116±72	0.0001	0.4
L2/L3 SAT (cm^2)	151±89	167±85	183±83	203±87	0.0001	0.5
L4/L5 VAT (cm^2)	68±44	76±43	89±50	101±51	0.0001	0.6
L4/L5 SAT (cm^2)	243±105	263±105	275±106	294±103	0.0001	0.9
L4/L5 SSAT (cm^2)	116±64	126±68	143±75	154±70	0.0001	0.8
L4/L5 DSAT (cm^2)	123±54	133±52	146±52	154±55	0.001	0.7
Liver density (HU)	58±5	55±4	51±16	48±16	0.0001	0.6
Fat cell size (µm)	58±7	58±5	60±6	59±8	0.5	0.8

349 VAT- visceral adipose tissue, SAT- subcutaneous adipose tissue, SSAT- superficial
350 subcutaneous adipose tissue, DSAT- deep subcutaneous adipose tissue

351 *Central fat was measured by DXA

352

Figure Legend:

Fig 1 Study timeline and food consumption regimen. From day -3 to the baseline study, all foods were provided to subjects at calculated baseline energy requirements (A; 30% fat, 15% protein and 55% carbohydrates). During the overfeeding phase (in grey), on days $0 - 3$ and $25 - 28$, all foods were provided to subjects at calculated baseline energy requirements + 1250 kcal/d (B; 45% fat, 15% protein and 40% carbohydrates). On days $3 - 25$, subjects were instructed to consume their regular diets and were provided with high fat snacks to provide additional 1250 kcal/day (C).

Fig 2 Weight, circulating hormones and metabolites, HOMA-IR and respiratory quotient at baseline and in response to overfeeding Weight (A), serum leptin (B), glucose (C), insulin (D), C-peptide (E), HOMA-IR (F), NEFA (G) and RQ (H) at baseline and at +3- and +28- days of overfeeding in subjects with (white squares) and without (black squares) a family history of type 2 diabetes. Difference from baseline $*p < 0.05$, $**p < 0.005$; difference between groups $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\delta}p = 0.06$.

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