Diabetologia (2010) 53:1700–1708
DOI 10.1007/s00125-010-1768-y

ARTICLE

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

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Received: 7 January 2010 / Accepted: 30 March 2010 / Published online: 12 May 2010

Abstract
Aims/hypothesis The purpose of the study was 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 5,200 kJ/day in 41 sedentary individuals with and without a family history of type 2 diabetes (FH+ and FH− respectively). Measures included body weight, fat distribution (computed tomography) and insulin sensitivity (hyperinsulinaemic–euglycaemic clamp).
Results Body weight was increased compared with baseline at 3 and 28 days in both groups ($p<0.001$), FH+ individuals having gained significantly more weight than FH− individuals at 28 days (3.4±1.6 vs 2.2±1.4 kg, $p<0.05$). Fasting serum insulin and C-peptide were increased at 3 and 28 days compared with baseline in both groups, with greater increases in FH+ than 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 a 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} [kg fat-free mass]$^{-1}$, $p=0.03$), and insulin sensitivity by HOMA-IR decreased at both time points ($p<0.001$) and to a greater extent in FH+ than in FH− ($p=0.008$). Liver fat, subcutaneous and visceral fat increased similarly in the two 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. The results of this study suggest that healthy individuals with a family history of type 2 diabetes are predisposed to adverse effects of overfeeding.

Trial registration: ClinicalTrials.gov NCT00562393
Funding: The study was funded by the National Health and Medical Research Council (NHMRC), Australia (no. #427639).

Keywords First-degree relatives · Insulin resistance · Liver fat · Overfeeding · Type 2 diabetes

Abbreviations

CT	Computed tomography
DXA	Dual energy X-ray absorptiometry
FFM	Fat-free mass
FH	Family history
FM	Fat mass
HOMA-IR	HOMA–insulin resistance
RMR	Resting metabolic rate
RQ	Respiratory quotient

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Introduction

Overnutrition and a 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 of isoenergetic 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 has previously been 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 [12] insulin sensitivity. 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 than in those without a history of type 2 diabetes (FH−).

Methods

Subjects Sedentary, non-smoking, non-diabetic men and women who reported either no family history of type 2 diabetes (FH−) or at least one first-degree relative with type 2 diabetes (FH+) were recruited by advertising in local newspapers. Subjects were excluded if their 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− participant 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 of the study.

Diets Estimated energy requirements were calculated for each participant 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 dietitian then planned individual menus for participants. Study timeline and food consumption regimen are outlined in Fig. 1. Briefly, from day −3 to day 0 all foods were provided at baseline energy requirements with a nutrient composition of 30% of energy as fat, 15% as protein and 55% as carbohydrate. From days 0–3 and 25–28, all foods were provided at baseline energy requirements plus 5,200 kJ/day with a nutrient composition of 45% fat, 15% protein and 40% carbohydrate. During the overfeeding phase we aimed to double the amount of fat intake by providing three high energy-high fat snacks per day, each providing ~1,000 kJ (e.g. potato crisps, chocolate bars, cheesecake) and a liquid oil-based supplement (Benecalorie; Novartis, Basel, Switzerland; ~1,400 kJ) mixed in a dairy dessert (~800 kJ). On days 3–25 of overfeeding, participants were instructed to consume their regular diets and were provided with the above snacks and supplement to achieve an intake of 5,200 kJ/day above the baseline energy requirement. They were required to complete a checklist every day, reporting which snacks were consumed, complete a 3 day diet diary once before study commencement and twice during the overfeeding phase, and to meet the study dietitian weekly. The checklists were reviewed during weekly weighings by the study dietitian so that any deviations from protocol were quickly identified and alternative options could be provided, in order 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, QLD, Australia). Thirty-two participants returned the diet diaries of both study phases (Table 1).

Metabolic testing Subjects attended the clinical research facility at 08:00 hours after a 12 h overnight fast at baseline

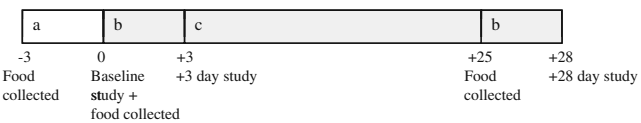

Fig. 1 Study timeline and food consumption regimen. From day −3 to the baseline study, all foods were provided to participants at calculated baseline energy requirements (a; 30% of energy as fat, 15% as protein and 55% as carbohydrate). During the overfeeding phase (shaded), on days 0–3 and 25–28, all foods were provided to participants at calculated baseline energy requirements +5,200 kJ/day (b; 45% of energy as fat, 15% as protein and 40% as carbohydrate). On days 3–25, participants were instructed to consume their regular diet and were provided with high-fat snacks to provide an additional 5,200 kJ/day (c)

Table 1 Diet diary analysis at baseline and during overfeeding by group

Component	Group				*p* value	
	FH−		FH+			
	Baseline	Overfeeding	Baseline	Overfeeding	Time	Group
Energy (kJ)	7,950±2,470	12,100±2,680	8,710±2,640	14,100±3,810	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

Data are based on n=32 (19 FH− and 13 FH+)

PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids, SF, saturated fatty acids

and on days 3 and 28 of overfeeding (Fig. 1). The baseline and 28 day visits were identical: weight, height and blood pressure were measured in a hospital gown after voiding and fasting blood samples were drawn. After 30 min of supine rest, resting metabolic rate (RMR) and respiratory quotient (RQ) were determined for 30 min (ParvoMedics, UT, USA). A peri-umbilical subcutaneous fat biopsy was performed as described previously [19]. Insulin sensitivity was then measured using a 2 h hyperinsulinaemic–euglycaemic clamp (60 mU m^{-2} min^{-1}), as described previously [10]. Glucose was infused at a variable rate to maintain glucose at 5.0 mmol/l and the steady-state glucose infusion rate was calculated between 90 and 120 min. Indirect calorimetry was repeated in conjunction with the steady-state measurement. Stanford 7 day activity recalls were administered at baseline and after 28 days of overfeeding according to previously published guidelines [20]. At 3 days of overfeeding, weight and blood pressure were measured, fasting blood samples were obtained and indirect calorimetry was performed. Participants attended the clinical research facility weekly for weight follow-up, snack collection and consultation with the study dietitian.

Body composition Fat mass, fat-free mass and central abdominal fat were assessed at baseline and day 28 of overfeeding by dual energy x-ray absorptiometry (DXA; Lunar DPX-Lunar Radiation, Madison, WI, USA), as described previously [21]. Three cross-sectional computed tomography (CT) scans (Gemini GXL; Philips, the Netherlands), 1 cm wide, centred on the L2–L3, L4–L5 and the T12–L1 disc spaces 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, as described previously [19]. CT images were analysed using Gemini (GXL Host System; Philips, the Netherlands). Two participants did not undergo CT scans. L4–L5 superficial and deep subcutaneous adipose tissues were not able to be analysed in four participants and the spleen was not visualised in two participants.

Measurement of fat cell size Subcutaneous adipose biopsies were fixed in Bouin's fluid, dehydrated, paraffin-embedded and then sectioned (4 μm). 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 was measured in 33 participants who had histological samples available before and after intervention. Adipocyte diameter was measured blindly and for at least two 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, OH, USA). Fasting serum insulin, C-peptide and leptin were assayed by radioimmunoassay (Linco Research, St Charles, MO, USA). HOMA-IR was calculated as described previously [22]. HDL-cholesterol and triacylglycerol were evaluated by enzymatic colorimetry (Roche, IN, USA). LDL was calculated by the Friedewald equation. NEFA was measured by an enzymatic colorimetry assay (Wako, Osaka, Japan).

Statistical analysis Data are presented as mean ± SD unless otherwise stated. Statistics were analysed with SPSS 15 (SPSS, Chicago, IL, USA). Leptin and insulin data were not normally distributed and were log-transformed for analysis. Baseline differences between groups were analysed by one-way ANOVA. All other data were 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 analysis 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 described previously [16]. Correlations

were calculated using Pearson's correlation coefficient. Significance was set at $p<0.05$.

Results

Baseline characteristics Baseline characteristics by group are shown in Table 2. There were no detectable differences between groups at baseline with respect to age, weight, BMI, blood pressure, fasting glucose, insulin, C-peptide, leptin, lipid profile or peripheral insulin sensitivity. The only difference was in carbohydrate oxidation in response to insulin infusion during the hyperinsulinaemic–euglycaemic clamp (ΔRQ), which was lower in FH+ than in FH− individuals. At baseline, peripheral insulin sensitivity was related to liver/spleen ratio ($r=0.5$, $p=0.001$) and visceral adipose tissue ($r=-0.4$, $p=0.01$). Fat cell size correlated with body fat percentage by DXA ($r=0.4$, $p=0.04$), subcutaneous adipose tissue ($r=0.4$, $p=0.02$), serum NEFA ($r=0.4$, $p=0.02$), triacylglycerol ($r=0.5$, $p=0.006$) and insulin resistance by HOMA-IR ($r=0.5$, $p=0.007$).

Diet diary and physical activity questionnaire analysis Reported dietary intakes at baseline and during overfeeding by group are given in Table 1. Dietary fat (g) approximately doubled in the overfeeding phase. Carbohydrate and protein intakes also increased ($p<0.0001$). There were no significant differences in energy, carbohydrate or protein intake between groups, although a tendency was noted for FH+ to consume more total energy ($p=0.15$) and more fat during overfeeding ($p=0.07$) compared with FH− participants. The average self-reported consumption of snacks during the overfeeding period was 92±14% and 95±9% in FH− and FH+ participants respectively ($p=0.4$). Reported levels of physical activity were similar at baseline (230±8 and 232±12 metabolic equivalent of task (MET-h week^{-1} in FH− and FH+ respectively, $p=0.7$) and did not change with overfeeding (229±7 and 231±7 MET-h week^{-1} in FH− and FH+ respectively; time $p=0.4$, group $p=0.8$).

Changes in weight and fat distribution in response to overfeeding As expected, overfeeding resulted in significant weight gain at 3 and 28 days compared with baseline (Fig. 2a). At 3 days, weight gain was not different between groups. At 28 days, FH+ individuals had gained 1.2 kg more than FH− individuals ($p<0.05$). Weight gained as percentage of baseline weight was 0.7±0.9% and 1.0±0.7% in FH− and FH+ respectively at 3 days ($p=0.4$) and 3.1±2.0% and 4.4±2.0% in FH− and FH+ respectively at 28 days of overfeeding ($p<0.05$). Fat mass, fat-free mass, central fat by DXA and visceral and subcutaneous adipose tissue volume by CT increased similarly in the two groups (Table 3). Circulating leptin increased significantly with weight gain and to a greater extent in FH+ than in FH− participants at 28 days (Fig. 2b), consistent with the greater weight gain in the former group. The increases in circulating leptin concentration at days 3 and 28 correlated with weight gains at these time points ($r=0.3$, $p=0.03$ at 3 days and $r=0.5$, $p=0.003$ at 28 days).

Table 2 Baseline characteristics of study participants

Characteristic	Group			*p* value
	Whole cohort	FH−	FH+	
Male/female	21/20	12/12	9/8	
Age (years)	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} [kg FFM]$^{-1}$)	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 (kJ/day)	5,800±930	5,600±1,070	6,040±680	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

GIR, glucose infusion rate

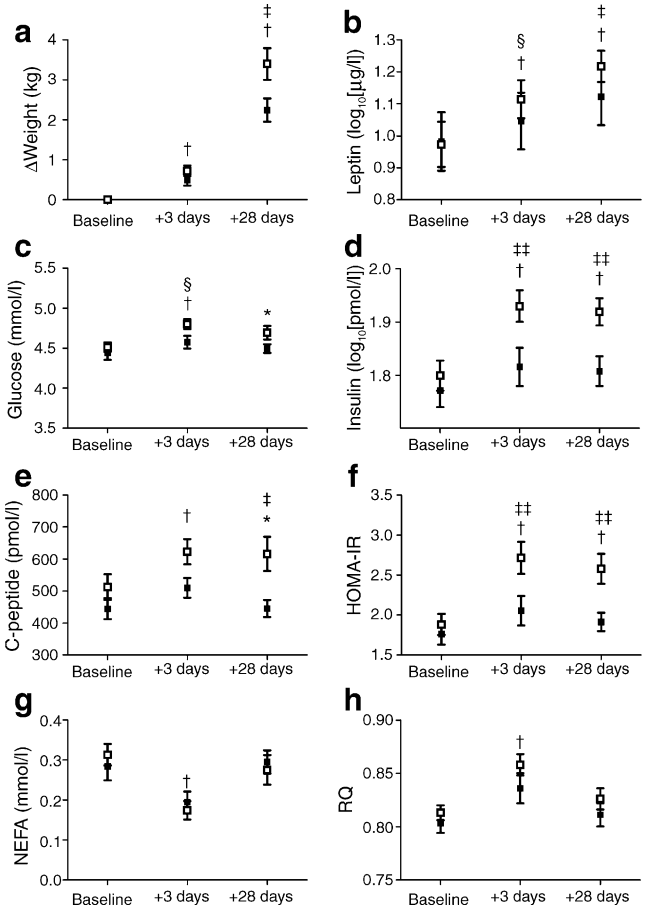

Fig. 2 Body weight, circulating hormones and metabolites, HOMA-IR and respiratory quotient at baseline and in response to overfeeding. Body weight (**a**), serum leptin (**b**), glucose (**c**), insulin (**d**), C-peptide (**e**), HOMA-IR (**f**), NEFA (**g**) and RQ (**h**) at baseline and at +3 and +28 days of overfeeding in participants with (white squares) and without (black squares) a family history of type 2 diabetes. Difference from baseline: $^{*}p<0.05$, $^{\dagger}p<0.005$. Difference between groups: $^{\ddagger}p<0.05$, $^{\ddagger\ddagger}p<0.01$, $^{\S}p=0.06$

Preferential fat gain in the L2–L3 visceral depot was observed in the whole cohort, increasing from 33±15% at baseline to 34±14% at 28 days of overfeeding ($p=0.008$). However, this was abolished when baseline visceral volume was included as a covariate ($p=0.1$). Liver fat increased significantly in response to overfeeding similarly in the two groups (Table 3) and correlated with weight gain ($R^2=0.17$, $p=0.01$). Abdominal fat cell size did not change (Table 3).

Metabolic responses to overfeeding Fasting glucose increased significantly at days 3 and 28 of overfeeding compared with baseline, but this response was not significantly different between groups (Fig. 2c). Fasting insulin and C-peptide increased at 3 and 28 days of overfeeding (Fig. 2d, e), with a greater increase in insulin in FH+ than in FH− individuals at both time points ($p<0.01$, Fig. 2d) and in C-peptide at 28 days ($p<0.05$; Fig. 2e). Accordingly, HOMA-IR (reflecting fasting insulin resistance) increased significantly in both groups ($p<0.005$ for both time points; Fig. 2f), the increase being more pronounced in FH+ individuals ($p<0.01$). Peripheral insulin sensitivity measured by the hyperinsulinaemic–euglycaemic clamp at 28 days decreased significantly in the whole cohort from 54.8±17.7 to 50.3±15.6 μmol min^{-1} [kg FFM]$^{-1}$ at 28 days of overfeeding ($p=0.03$), but was not different between groups. Total cholesterol and HDL-cholesterol increased significantly in response to overfeeding similarly in the two groups (4.6±1.0 to 4.8±1.0, $p=0.01$ for total cholesterol and 1.3±0.3 to 1.4±0.4, $p<0.0001$ for HDL-cholesterol). 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 the basal level at 28 days of overfeeding, without a group effect ($p=0.4$; Fig. 2g). Plasma NEFA was suppressed in the hyperinsulinaemic state (by clamp) at both baseline and 28 days of overfeeding ($p<0.0001$), without a group difference at both time points (data not shown). Similarly, fasting RQ increased at 3 days ($p<0.0001$) and returned to the basal level at 28 days, without a group effect ($p=0.8$; Fig. 2h). The ΔRQ during the clamp was not altered by overfeeding (data not shown). Absolute RMR at baseline was not different between groups (Table 2) and increased after overfeeding at 3 and 28 days in both groups ($p<0.01$). This increase was not different between FH− and FH+ groups at 3 days (290±650 and 350±550 kJ/day respectively, $p=0.8$) or 28 days (330±710 and 350±550 kJ/day respectively, $p=0.9$). 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 described previously [16], and found no difference between the predicted and measured RMR at 28 days of overfeeding (data not shown). We did not perform DXA measurements at day 3 but repeated this analysis using body weight at baseline (RMR=344.9+14.0×body weight). Similarly, we found no difference at 28 days. However, at day 3, measured RMR (6,100±990 kJ/day) was significantly elevated above weight-predicted RMR (5,800±640 kJ/day, $p=0.03$). There was no difference between groups in this response (data not shown). The increase in thermogenesis in response to insulin infusion was 480±690 kJ/day at baseline and 520±620 kJ/day after overfeeding; neither value differed between groups (data not shown).

Discussion

High-fat overfeeding induces insulin resistance in rodent models in as little as 3 weeks [23, 24]. In this study we established that 28 days of overfeeding induced weight gain

Table 3 Body weight, body fat percentage and central fat (by DXA), abdominal fat distribution and liver density (by CT) and fat cell size at baseline and after 28 days of overfeeding in participants with or without a family history of type 2 diabetes

Variable	Group				*p* value	
	FH−		FH+			
	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)[a]	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 (Hounsfield units)	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

[a] Central fat was measured by DXA

DSAT, deep subcutaneous adipose tissue; SAT, subcutaneous adipose tissue; SSAT, superficial subcutaneous adipose tissue; VAT, visceral adipose tissue

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 reported 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+ than in FH− individuals. Moreover, since overconsumption by 44 kJ is required to gain 1 g of body weight [25, 26] 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 weight 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 compared with FH− individuals. This outcome is of great interest as identical dietary instructions and food options were provided to both groups. Although FH+ had gained on average 1.2 kg more than FH− individuals at day 28, we did not detect significant group differences in compartmental gain by CT and DXA. This apparent inconsistency may stem from the lower reproducibility and higher variability of DXA [27] and CT estimates compared with a single scale weight. Interestingly, we have previously reported impaired peptide YY secretion in response to a meal in FH+ individuals [28], which may contribute to reduced satiety and facilitate weight gain. Even the classical experimental overfeeding studies [29–31], in which participants were incarcerated and all foods were provided, have shown wide variability in weight gain in response to controlled overfeeding. Furthermore, an experimental weight gain study in twins demonstrated a heritable component of weight gain, with much greater variability in weight gain between than within twin pairs [31]. In those studies, since energy intake was very carefully controlled, it is likely that differences in weight gain were due to variations in the thermogenic response to overfeeding and possibly non-exercise activity thermogenesis [32]. In the present study, we suggest 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 [33] in healthy lean men. Our initial hypothesis was that metabolic defects associated with overfeeding would be greater in FH+ than in FH− individuals. Indeed, we observed that fasting serum insulin and C-peptide increased more in individuals with a family history of type 2 diabetes than in those without such a family

history. Fasting blood glucose tended to increase more in FH+ than in FH− individuals at +3 days of overfeeding; this may account for the significant increase in insulin in the FH+ 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 an IVGTT [12]. In longer term overfeeding studies, reduced insulin clearance was observed after 4.5 months and significant weight gain in lean young men [33]. These findings are similar to those in dogs fed a diet with a moderately increased fat level, in which an increase in beta cell secretion was observed at 6 weeks but was no longer evident at 3 months, when hyperinsulinaemia was maintained by reduced insulin clearance by the liver [34].

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, 35]. 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 and CT measurement of liver fat content are closely correlated [36]. 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 high fat overfeeding has been shown in rodents and dogs [37, 38] and in response to 3 days of a high fat overfeeding in healthy lean men by magnetic resonance spectroscopy [39]. Interestingly, moderate energy restriction for 2 days decreases liver fat in obese people [40]. Increased visceral fat is also closely associated with insulin resistance [41] and increased visceral adipose tissue is observed in BMI-matched insulin-resistant FH+ individuals [6]. In the present study, visceral adiposity was similar between groups at baseline and FH+ individuals were not more likely than FH− individuals to deposit fat in the visceral compartment in response to overfeeding. Increased fat cell size is also observed in insulin resistance and may represent failure of the adipose tissue mass to expand to accommodate increased energy intake [19]. We did not detect an increase in average fat cell size with the moderate weight gain achieved in this study. This result is in contrast to the results of a historical experimental overfeeding study [29], but that intervention was longer and weight gain was much greater.

There is some evidence to suggest that post-obese individuals do not appropriately oxidise dietary fat, which may predispose them to weight regain [42]. There is also marked variability between individuals in the capacity to switch appropriately between fat and carbohydrate oxidation [43]. This response has been termed metabolic flexibility [44] 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+ individuals 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 the increased weight gain observed in FH+ individuals. Overfeeding initially suppressed fatty acid oxidation. This may have been 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. The results of this study suggest 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 the development of type 2 diabetes in an obesogenic environment.

Acknowledgements We would like to thank L. Schofield (the study co-ordinator) and A. Jenkins for statistical advice, and all the volunteers who participated in this demanding research study. The study was funded by the National Health and Medical Research Council (NHMRC), Australia. L. K. Heilbronn is supported by an NHMRC Career Development Award (No. 481354) and C. S. Tam by an NHMRC/NHF Postgraduate Biomedical Scholarship (No. 457224).

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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