Modest changes to glycemic regulation are sufficient to maintain glucose fluxes in healthy young men following overfeeding with a habitual macronutrient composition

Dale J. Morrison[1], Greg M. Kowalski[1], Clinton R. Bruce[1], Glenn D. Wadley[1].

1. *Deakin University, Geelong, Australia, Institute for Physical Activity and Nutrition (IPAN), School of Exercise and Nutrition Sciences*

Running title: Overfeeding and postprandial glucose flux

4705 words

2 Tables; 4 Figures

Address for correspondence: Glenn Wadley,

ORCID identifier 0000-0002-6617-4359

Institute for Physical Activity and Nutrition

School of Exercise and Nutrition Science

Deakin University

221 Burwood Highway

Burwood, Australia

3125

Email: glenn.wadley@deakin.edu.au

Abstract

Currently, it is unclear whether short-term overfeeding in healthy people significantly affects postprandial glucose regulation, as most human overfeeding studies have utilized induced experimental conditions such as the euglycemic-hyperinsulinemic clamp technique to assess glucoregulation. The aim of this study was to quantify glucose fluxes (rates of meal glucose appearance (Ra), disposal (Rd) and endogenous glucose production (EGP)) in response to 5 and 28 days of overfeeding (+45% energy) while maintaining habitual macronutrient composition (31.0 ± 1.9 % fat; 48.6 ± 2.2 % carbohydrate; 16.7 ± 1.4 % protein) in healthy, lean young men. Meal tolerance testing was combined with the triple-stable isotope glucose tracer approach. Visceral adipose volume increased by ~15% with 5 days of overfeeding while there was no further change at 28 days. In contrast, body mass (+1.6kg) and fat mass (+1.3kg) were only significantly increased after 28 days of overfeeding. Fasting EGP, Rd and insulin were increased at 5, but unchanged after 28 days. Postprandial glucose and insulin responses were unaltered by 5 days of overfeeding, but were modestly increased after 28 days ($P<0.05$). However, meal Ra and glucose Rd were significantly increased after both 5 and 28 days of overfeeding ($P<0.05$). Despite this, overfeeding did not lead to alterations to postprandial EGP suppression. Thus, in contrast to findings from euglycemic-hyperinsulinemic clamp studies, chronic overfeeding did not affect the ability to suppress EGP or stimulate Rd under postprandial conditions. Rather, glucose flux was appropriately maintained following 28 days of overfeeding through modest increases in postprandial glycemia and insulinemia.

The past few decades have seen an immense rise in both obesity and type 2 diabetes (18). Considering their rapid worldwide development, it is unlikely that this has been driven by genetics alone. Rather, it is evident that lifestyle factors, such as the broad availability of inexpensive, highly palatable energy dense foods are playing a significant role in this epidemic (15, 49). This widespread overconsumption of energy dense foods, particularly in the form of processed starch, sugar and fat (8, 49) is characteristic of the Westernised diet, and is likely to be a crucial factor leading to the development of insulin resistance and glucose intolerance (10). Considering that overfeeding in humans, even in the short-term (1-7 days), can impair glycemic control and insulin action (14, 22, 28, 34, 35, 38), understanding the processes governing these overfeeding-induced changes may help provide insight into metabolic disease progression.

Short-term experimental overfeeding is a model often used in animal studies to replicate overconsumption in humans, and these studies have consistently demonstrated that defects in hepatic glucose metabolism occur within a few (1-7) days of the onset of overconsumption, preceding the induction of peripheral defects which take several weeks to emerge (24, 27, 33, 46). Although previous human studies have also observed a similar temporal pattern of glucoregulatory defects, such as rapid development of impaired hepatic glucose metabolism in response to overfeeding (10, 15, 40, 41), our current understanding of the physiological implications of overfeeding in humans is complicated by a number of factors.

Firstly, many human overfeeding trials use experimental diets that not only provide energy excess, but also alter the composition of macronutrients, favoring an increase in the amount of energy derived from fat (10, 12, 22, 34, 35, 38). Indeed, many of the recent human studies reporting that overfeeding alters glucose metabolism have also provided a high proportion of fat, often greater than 40% of energy intake (10, 12, 22, 34, 38, 39). Therefore, it is not clear if the observed impairments in glucoregulation are a transient pathological result of the high

fat content of the diets, an increase in total energy intake or simply due to the increased dietary fat availability. Furthermore, it is unlikely that overfeeding one specific macronutrient (i.e. fat) is actually representative of a real life overeating paradigm. The typical fat composition of diets in most Western countries is ~30-35% energy (1, 42, 48), and it is likely that consumption of energy dense foods provides excess of carbohydrate and protein, along with fat.

Additionally, many human studies investigating glucose metabolism following periods of overfeeding utilize experimental techniques such as the hyperinsulinemic clamp (2, 10, 12, 15, 28, 35, 38) or intravenous glucose tolerance test (IVGTT) which are not truly representative of postprandial conditions (10). Importantly, those studies suggesting that rapid induction of hepatic defects in humans occur within 7 days of overfeeding have exclusively utilized steady-state techniques (10, 15, 40, 41). While some human overfeeding studies have utilised glucose or mixed meal tolerance tests to assess glycemic control (14, 22, 34), to the best of our knowledge none have concomitantly utilised glucose isotope tracers to determine glucose fluxes under these conditions.

Thus, while it is clear that overfeeding for as little as 3-7 days in humans can lead to increased fat mass and impaired glycemic regulation under experimental steady state conditions (2, 10, 12, 15, 22, 28, 34, 46), the effects of overfeeding on postprandial glycemic regulation, specifically regarding glucose fluxes, is not well characterized. Therefore, our aim was to examine the effects of overfeeding, independent of changes in habitual macronutrient composition, on postprandial glucose metabolism. Specifically, we hypothesized that impairments to postprandial EGP suppression could occur, given that in humans as little as 5 days of overfeeding has been previously shown to impair EGP suppression during the hyperinsulinemic clamp (24, 27, 33, 46). Accordingly, we utilized mixed meal tolerance testing combined with the variable infusion triple-stable isotope glucose tracer approach (i.e.

tracer clamp (4, 32, 45)) to assess postprandial glucose fluxes (glucose appearance, production and disposal) following both short-term (5 days) and chronic (28 days) overfeeding in healthy lean young adult males.

Methods

Participants

Eight young healthy men participated in the study. Participant characteristics are presented in Table 1. Exclusion criteria included those currently diagnosed with, or with a family history of diabetes, taking medications or herbal supplements, BMI >30 $kg.m^2$, smoking, engaging in structured exercise >90 min per week or being non weight-stable for at least 6 months.

Experimental design

Participants arrived at the clinical research facility at 0700 h in the overnight (10 h) fasted state, having refrained from exercise and alcohol consumption for 48 h. For the 24 h prior to the baseline experimental trial, participants were provided with an energy maintenance diet (9,783 kJ, 55% carbohydrate, 30% fat, 15% protein) designed to be representative of the typical macronutrient composition of an Australian diet (40). A 22-gauge cannula was inserted into a vein of each forearm for tracer infusions and venous blood sampling, respectively. Sterile stable isotopes; [1-^{13}C] glucose; [6,6-^{2}H] glucose; and [U-^{13}C] glucose (Cambridge Isotope Laboratories, Andover, MA, USA) were prepared as previously described (32).

Triple tracer protocol

Experimental trials occurred as we have previously detailed (32). Briefly, a primed-continuous intravenous infusion of [1-^{13}C] glucose was initiated and continued until the end of the study, where a bolus of 33.3 $\mu mol.kg^{-1}$ was infused over 5 min followed by a constant infusion (0.333 $\mu mol.kg.min^{-1}$) for the following 150 min. Blood samples were taken during the equilibration period at designated time points; -150 (immediately before infusion), -60, -30, -20, -10 and 0 min. All basal and postprandial blood samples were immediately spun in a centrifuge at 3000g for 15 min at 4°C and plasma was stored at -80°C until analysis.

At the designated time point 0 min, participants ingested a mixed meal (10 kcal.kg^{-1}, 45% carbohydrate, 20% protein, and 35% fat) consisting of eggs, cheese and 1.2 g.kg^{-1} glucose (including [6,6-^{2}H] glucose at an enrichment of 4% w/v) in sugar-free Jell-O (Aeroplane Jelly, Victoria, Australia) as the sole carbohydrate source. The meal was consumed within 10 min. At 0 min (i.e. with the first bite), the infusion of [1-^{13}C] glucose was altered in a pattern so as to approximate the anticipated fall in EGP (0-10min = 70%; 10-20min = 60%; 20-30min = 50%; 30-180min = 35%; 180-210min = 40%; 210-270min = 50%; percent of the basal rate of 0.333 μmol.kg.min^{-1}). At the same time, an infusion of [U-^{13}C] glucose was started (1.11 μmol.kg.min^{-1}) and the rate varied to mimic the anticipated appearance of [6,6-^{2}H] glucose from the meal (0-10min = 25%; 10-30min = 100%; 30-70min = 65%; 70-90min = 55%; 90-120min = 45%; 120-150min = 35%; 150-180min = 25%; 180-210min = 20%; 210-270min = 10%; percent of the maximal infusion rate of 1.11 μmol.kg.min^{-1}). Blood samples were taken at 10, 20, 30, 40, 50, 70, 90, 120, 150, 180, 210, and 270 min after meal ingestion.

Participants underwent three identical experimental trials, at baseline, and the morning after completing both short-term (5 days) and chronic (28 days) overfeeding. Body composition, including fat-free mass, fat mass and visceral adipose volume, were determined using a Lunar Prodigy whole-body DEXA scanner (GE Medical Systems, Madison, WI) in total body scan mode (36).

Overfeeding protocol

All participants completed 28 d of overfeeding. During this period, participants were instructed to consume their regular diets and were provided with snacks to achieve an energy intake of ~5,000 kJ/day above their baseline energy requirements. The overfeeding diet was designed to maintain the macronutrient composition at ~53% carbohydrate, 32% fat and 15%

protein, to be representative of a typical Australian dietary macronutrient composition (42). The snacks included energy-dense foods such as chips, chocolate and meal replacement shakes. For the 24 h preceding the short-term and chronic overfeeding mixed meal tolerance testing trials, all foods were provided at baseline energy requirement plus additional ~5000 kJ/day, with a nutrient composition of 55% carbohydrate, 30% fat and 15% protein.

Participants were required to complete daily checklists during the overfeeding period, indicating which snacks were consumed and to complete 3-day diet diaries three times throughout the trial, from days -3 to 0, 2 to 5, and 25 to 28, as well as Stanford 7 day activity recalls at baseline and after 28 days of overfeeding according to previously published guidelines (31). Participants visited the lab weekly to monitor bodyweight, return food diaries, dispensation of snacks and review of checklists so that any deviations from the protocol were quickly identified and corrected. At the end of the study, diets were analysed for macronutrient composition using Foodworks 2007 based on the Australian foods database (Xyris Software, QLD, Australia).

Plasma hormones and metabolites

Plasma obtained from EDTA-containing tubes was used for insulin and C-peptide determination via sandwich ELISA assay (Insulin, ALPCO, NH, USA; C-Peptide, EMD Millipore, MA, USA). Plasma non-esterified fatty acids (NEFA) (NEFA C; Wako Chemicals, VA, USA) and triglycerides (TAG) (GPO-PAP reagent, Roche Diagnostics, Basel, Switzerland) were determined by colorimetric assays. Plasma obtained from lithium-heparin containing tubes was used to determine plasma glucose concentration by the glucose oxidase method.

Analysis of tracer enrichment

Tracer enrichment in plasma, infusates and labelled meal samples was measured using methane positive chemical-ionization gas chromatography-mass spectrometry (GC-MS). Preparation of the glucose aldonitrile pentapropionate derivative was undertaken as described by Antoniewicz et al. (3). Briefly, 10 µl of plasma was mixed with 100 µl of ice-cold analytical grade methanol and centrifuged for 5 min to precipitate plasma protein. The supernatant (~90 µl) was removed and evaporated to dryness in glass GC inserts under vacuum at 40°C using a centrifugal speed evaporator. Dried samples were dissolved in 50 µl of hydroxylamine hydrochloride solution (20 mg/mL in pyridine) and heated at 90°C for 60 min after which 100 µL of propionic anhydride was added. Following 30 min incubation at 60°C, samples were dried at 40°C under vacuum as described above and dissolved in 100 µL of ethyl acetate for subsequent analysis via GC-MS.

Samples were injected using a 1:20 split ratio onto a HP-5MS 5 % Phenyl Methyl Siloxane column (30.0 m x 250 µm x 0.25 µm; Agilent technologies, Santa Clara, CA, USA) connected to an Agilent 7890B Gas Chromatograph. Target compounds were detected using an Agilent 5977B Mass Selective Detector (MSD). The GC program consisted of a 35 °C/min ramp starting at 60 °C. A final temperature of 280 °C was then held for three minutes. Helium was used as the carrier and methane as the reagent gas. The MSD was operated in the selected ion monitoring mode measuring the intact (C1-C6) molecular ions at mass to charge ratios (m/z; M0-M+6) 384, 385, 386, 387, 388, 389 and 390, corresponding to natural unlabelled (384, M0), [1-^{13}C] (385, M+1) [6,6 - ^{2}H] (386, M+2) and [U-^{13}C] (390, M+6) glucose. Ion abundances were quantified using the Mass Hunter Workstation (Agilent Technologies, Santa Clara, CA, USA). The raw isotopomer data were corrected for natural isotopic background abundance skew using the matrix method (30), permitting enrichments to be expressed as mole percent excess.

Calculations

Glucose fluxes, meal rate of appearance (Ra), rate of disposal (Rd) and EGP were calculated using Steele's non-steady state model (43) as described in detail previously (32). Insulin secretion rate was calculated using glucose and C-peptide kinetics in a computerised program implementing a regularisation method of deconvolution (20). Hepatic insulin extraction was calculated as insulin secretion rate AUC/plasma insulin AUC (9). The area under the curve (AUC) for glucose, insulin, C-peptide, NEFA, TAG and insulin secretion rate was calculated using the trapezoidal method. Fasting glucose, Rd and EGP are reported as the average of time points -150, -60, -30, -20 and -10 and 0 min. Fasting insulin and C-peptide are reported as the average of time points -150 and 0 min.

Statistical analysis

Differences between baseline, short-term overfeeding and chronic overfeeding were assessed with either a paired t-test, one-way or two-way repeated-measures ANOVA. Bonferroni post-hoc analysis was used to examine time-course differences between the conditions (i.e. baseline vs. short-term overfeeding; baseline vs. chronic overfeeding). All statistical analyses were performed using GraphPad Prism (version 6.0; La Jolla, CA, USA). All data are presented as mean ± SEM. Significance was set at $P<0.05$.

A priori power calculations were undertaken for the primary outcome measure EGP, as well as Rd. Due to the small number of triple tracer studies in humans assessing lifestyle interventions, the calculations were based on previous rodent studies demonstrating that hepatic and peripheral insulin sensitivity can decrease by 20-40% following overfeeding (27, 33, 46), and previously published human triple tracer data for typical EGP and Rd values (32). To detect a conservative 25% decrease in total EGP suppression (1747 ± 306) with 80% power, a sample size of 8 was required. To detect a conservative 20% decrease in glucose Rd AUC (6923 ± 806) with 80% power, a sample size of 6 was required.

214 Results

215 *Participant characteristics*

216 Participants consumed 4938 ± 87 kJ of additional energy to that normally supplied by their
217 regular diet, which equated to an additional 46% total energy intake (Table 1). Participants
218 achieved 96% and 98% compliance for consuming provided overfeeding snacks, for 5 and 28
219 days respectively. Dietary energy and macronutrient content derived from the participants'
220 normal diet was not significantly altered from baseline. Total dietary fat, carbohydrate and
221 protein intake significantly increased by overfeeding, while the percentage of energy derived
222 from these macronutrients did not change. While short-term overfeeding had little effect on
223 body composition (Table 1), body mass and fat mass were significantly higher after 28 days
224 of overfeeding than at baseline (1.64 ± 0.40 kg, $P<0.05$; 1.32 ± 0.18 kg, $P<0.05$, respectively,
225 Table 1). Both short-term and chronic overfeeding significantly increased visceral fat volume
226 by 59.5 ± 2.0 and 70.1 ± 2.7 $g.cm^{-2}$, respectively ($P<0.05$, Table 1).

227

228 *Plasma metabolites and hormones*

229 Fasting glucose, C-peptide, NEFA and TAG were unaltered by short-term overfeeding,
230 although fasting insulin was significantly increased compared to baseline (Table 2).
231 Compared to the glycemic responses at baseline, short-term overfeeding did not result in a
232 significant change to the postprandial glucose excursion (Figure 1A). Although short-term
233 overfeeding significantly increased plasma insulin and C-peptide levels at 30 min compared
234 to baseline (diet x time interaction, $P<0.05$, Figure 1B & C), this did not result in a significant
235 alteration to the postprandial insulin or C-peptide AUC (Table 2). Additionally, while insulin
236 secretion rate (Figure 1D) was unaltered, insulin clearance tended to decrease ($P=0.060$)
237 following short-term overfeeding (0.163 ± 0.02 vs. 0.128 ± 0.0.01 $l.min^{-1}.m^{-2}$; baseline vs.

short-term overfeeding, respectively). Postprandial NEFA (Figure 1E) and TAG (Data not shown) were not altered by acute overfeeding.

In regards to chronic overfeeding, the fasting glucose, insulin, C-peptide, NEFA and TAG were unaltered compared to baseline (Table 2). However, chronic overfeeding significantly increased the integrated postprandial glucose AUC from 0-120 min (Table 2) but not the total AUC for glucose. Additionally, the postprandial insulin AUC from 0-120 min was significantly increased (Table 2), while the pattern also differed such that there was a biphasic response following chronic overfeeding. Chronic overfeeding also significantly increased plasma C-peptide levels at 90 min (Figure 2C), but did not significantly alter the C-peptide AUC (Table 2). Both insulin secretion rate (Figure 2D) and insulin clearance (0.163 ± 0.023 vs. 0.138 ± 0.020 $l.min^{-1}.m^{-2}$; baseline vs. chronic overfeeding, respectively, P=0.179) were not significantly altered by chronic overfeeding. Postprandial NEFA (Figure 2E) and TAG (Data not shown) were not altered by chronic overfeeding.

Tracer-to-tracee ratios

Tracer-to-tracee ratios for [1-^{13}C] glucose to endogenous glucose (for calculation of EGP), and [U-^{13}C] glucose to [6,6-^{2}H] glucose (for calculation meal Ra) were maintained within a relatively narrow range, with the overall change over time less than 2-fold (Figure 3).

Glucose fluxes

Short-term overfeeding significantly increased fasting rates of EGP (10.9 ± 0.8 vs. 11.5 ± 0.8 μmol/kg/min; P<0.05 baseline vs. short-term overfeeding, respectively; Figure 4C) and glucose Rd (11.2 ± 0.8 vs. 11.9 ± 0.8 μmol/kg/min; P<0.05 baseline vs. short-term overfeeding, respectively; Figure 4B). While postprandial EGP suppression was unaltered by

short-term overfeeding (Figure 4C), both meal glucose Ra and glucose Rd were significantly increased over the initial 90 min of the postprandial period (Figure 4A; B). Consequently, there was a significant increase in both the total Ra AUC (5724 ± 334 vs. 6382 ± 271 μmol/kg, $P<0.05$ baseline vs. short-term overfeeding, respectively) and 0-120 min Ra AUC (3450 ± 315 vs. 4321 ± 364 μmol/kg, $P<0.05$), as well as the total Rd AUC (7328 ± 426 vs. 8036 ± 388 μmol/kg, $P<0.05$) and 0-120 min Rd AUC (4209 ±.337 vs. 5057 ± 391 μmol/kg, $P<0.05$).

Chronic overfeeding did not alter fasting EGP or Rd, nor the postprandial suppression of EGP compared to baseline (Figure 4F). Compared to baseline both meal glucose Ra and glucose Rd were significantly increased following chronic overfeeding at 20 and 30 min (Figure 4D; E). While the 0-120 min Rd (4209 ± 337 vs. 4785 ± 305 μmol/kg; $P<0.05$ baseline vs. chronic overfeeding, respectively) and Ra (3450 ± 315 vs. 4027 ± 289 μmol/kg, $P<0.05$) AUC was increased by chronic overfeeding, the total postprandial AUC for Rd and Ra was unaltered.

Meal and endogenous glucose

Following short-term overfeeding, plasma meal-derived glucose concentration was modestly, but significantly higher compared to baseline at 20 and 30 min (diet x time interaction, Figure 5A), although this did not translate into a significant change to the total integrated (689 ± 84 vs. 683 ± 66 mmol/l) or 0-120min meal glucose AUC (293 ± 27 vs. 333 ± 30 mmol/l). However, in regards to chronic overfeeding, meal-derived glucose concentration was significantly higher compared to baseline at 20, 40, 70, 90 and 120 min (diet x time interaction, Figure 5C), and this was associated with a significant increase to the total integrated (689 ± 84 vs. 790 ± 73 mmol/l, $P<0.05$ baseline vs. chronic overfeeding,

287 respectively), and 0-120min integrated meal glucose AUC (293 ± 27 vs. 394 ± 30 mmol/l,
288 $P<0.05$). The concentration of endogenous glucose was not significantly altered by short-term
289 or chronic overfeeding (Figure 5B; D).

Discussion

Our findings show that both short-term (5 d) and chronic (28 d) overfeeding in healthy young males, independent of changes in macronutrient composition, elicit only modest alterations to body composition, glycemia and insulinemia, and, in direct contrast to our hypothesis, no change to the pattern and magnitude of postprandial EGP suppression. However, both short-term and chronic overfeeding significantly increased meal glucose Ra, and while glucose Rd closely matched this rise in Ra, only chronic overfeeding demonstrated a significant, albeit small increase in postprandial glycemia. Interestingly, the change in plasma insulin occurred despite no change in insulin secretion rate, suggesting that the modest increase in glycemic and insulinemic excursions following chronic overfeeding likely permits more efficient stimulation of glucose flux without the need to drive a large change in compensatory beta cell insulin secretion.

An important finding of the current study was that fasting glucose was unaltered by overfeeding, and postprandial glycemia was only modestly increased by chronic, but unchanged by short-term overfeeding. This is in contrast to a range of previous data in humans, demonstrating that overfeeding for 3-7 days increases fasting (10, 12, 13, 22, 34) and postprandial (22, 34) glycemia in healthy humans. However, the majority of these overfeeding studies utilized diets which substantially increased the relative amount of energy derived from fat (10, 12, 22, 34). Indeed, following only 7 days of overfeeding a diet containing 60% energy from fat (increased from 31.5% in the habitual diet), Parry et al (34) recently demonstrated similar increases in postprandial glucose AUC and insulin AUC during a meal tolerance test as in the current chronic overfeeding study. Thus, 7 days of high fat overfeeding has a similar impact on postprandial glucose and insulin as 28 days of overfeeding with a mixed macronutrient composition. While some studies utilizing habitual macronutrient compositions or high-carbohydrate overfeeding diets have demonstrated

316 significant alterations to fasting or postprandial glycemia in the short-term (13), the changes
317 are typically much smaller than those from diets utilizing a high fat composition (2, 28).
318 Thus, overfeeding with a diet that increases the proportion of dietary fat may bias toward an
319 impairment in glucoregulatory function by increasing reliance on fat metabolism at the
320 expense of carbohydrate metabolism, more rapidly altering glucoregulatory function.

321 The macronutrient composition of overfeeding is also a key consideration in regards to
322 alterations to body composition. The relatively modest 1.2 kg increase in fat mass after 28
323 days of overfeeding in the current study compared to previous studies is likely a function of
324 the habitual macronutrient composition of the study diet. Indeed, previous studies (19, 29)
325 have demonstrated that 2-3 weeks of overfeeding with high-fat diets produces significantly greater
326 adipose tissue accumulation compared to overfeeding with carbohydrate –based diets.. This
327 heterogeneity in fat storage responses between fat and carbohydrate-based diets was shown to
328 occur as a result of progressive increases in total energy expenditure and carbohydrate
329 oxidation following high-carbohydrate overfeeding (19). Thus, greater carbohydrate
330 oxidation offsets the increase in energy intake and minimized nutrient storage compared to a
331 high-fat diet (19), highlighting the potent metabolic effect of consuming a high-fat diet,
332 above that of energy excess with an alternate macronutrient composition.

333 Increases in total body fat are strongly linked with metabolic disease progression (21), and
334 this is especially true for increase in visceral fat (17, 47). Interestingly, in the current study
335 visceral adipose volume was increased after both short-term and chronic overfeeding, despite
336 no change in body weight or fat mass after 5 days. However, despite the similar change to
337 visceral adipose volume after 5d and 28d of overfeeding, the postprandial glycemic response
338 was only increased following chronic overfeeding. On the other hand, Knudsen et al. (25)
339 recently demonstrated that decreased insulin sensitivity as assessed by Matsuda index and
340 euglycemic hyperinsulinemic clamp, and increased insulin during an OGTT occurred in

response to combined overfeeding and inactivity before the any change to visceral adipose tissue volume. Taken together, these findings suggest that the initial steps in the development of disturbed glucoregulatory function are not necessarily linked to visceral fat accumulation (25, 44).

In addition to increased postprandial glycemia, the systemic insulin response was increased following chronic overfeeding, suggesting some degree of insulin resistance. Modest changes in C-peptide during the meal tolerance test suggest that the accentuated insulin response following chronic overfeeding may be related to changes to insulin secretion. However, changes to insulin secretion also appear to be at best minimal, since the pattern and integrated response of modelled insulin secretion was not altered following overfeeding. Additionally, while modelled insulin clearance was not significantly altered following chronic overfeeding, it is possible that the current study was underpowered to detect modest changes in model-derived variables. Considering that insulin clearance was significantly decreased by ~20% following acute overfeeding, this suggests that decreased insulin clearance may occur in response to overfeeding as a mechanism to allow an appropriate degree of insulin into the periphery without placing additional burden on the beta cell. In reality, a combination of modest changes to both secretion and clearance likely explain the altered insulin response, as previous studies have shown that significant changes in body weight and insulin resistance increase both the postprandial insulin secretion, as well as insulin clearance rate in order to maintain glucose homeostasis in overweight and obese subjects (37).

In regards to postprandial glucose fluxes, it is possible that the increased postprandial glycemia following chronic overfeeding occurred as a mechanism for glucose itself to stimulate Rd considering that the mass effect of glucose to stimulate its own uptake and suppress its own production is a determinant of glucose tolerance (6, 26). Peterson et al. (35) recently demonstrated that the early stages of overfeeding-induced alterations to

glucoregulatory function appear to be driven more by declines in non-oxidative rather than oxidative glucose metabolism and it can be hypothesised that hyperglycemia may serve to compensate for the defects in non-oxidative disposal. Furthermore, the need to stimulate increased glucose Rd following both short-term and chronic overfeeding occurred in response to an increase in the systemic meal glucose Ra, suggesting that overfeeding may, at least transiently, lead to an adaptation in splanchnic tissues. Although speculative, the possible mechanisms that may serve to explain the increased meal glucose Ra in response to overfeeding include increased gastric emptying and/or reduced hepatic extraction of glucose. Considering that the rate of gastric emptying has a direct effect on the rate of glucose appearance after a meal (11), and that gastric emptying is accelerated early in the development of type 2 diabetes (16), this offers a possible explanation for the increased meal Ra following short-term and chronic overfeeding in the current study.

The observed increase in fasting rates of EGP in response to short-term overfeeding in the current study are consistent with previous findings in humans which have observed that development of impaired hepatic glucose metabolism occurs rapidly in response to overfeeding (10, 15, 40, 41). However, in contrast to this prevailing view, neither short-term nor chronic overfeeding was associated with a reduction in the postprandial suppression of EGP, and it is important to consider that previous studies have utilised steady-state measures of EGP, during fasting (10, 13, 40) or hyperinsulinemic clamp (15) conditions. Under postprandial, non-steady state conditions, rates of glucose flux are governed by the integrated regulation of β-cell insulin secretion, insulin sensitivity and glucose effectiveness (26). A number of researchers have demonstrated that effective compensation by these postprandial regulatory mechanisms can normalise postprandial suppression of EGP to maintain normal postprandial glucose concentrations in the face of insulin resistance (5, 7, 23). Thus, despite increased fasting rates of EGP in response to short-term overfeeding in the current study,

postprandial suppression of EGP was not reduced, likely due to effective compensation by portal vein hyperinsulinemia and hyperglycemia.

Another important observation in the current study is that fasting glucose was unchanged after 5d of overfeeding despite increased fasting EGP. This occurred concomitantly with an increase in fasting insulin suggesting the development of hepatic insulin resistance, although fasting Rd was also increased. Schwarz et al. (40) also observed increased fasting EGP in response to 5 days of carbohydrate overfeeding, although this also induced secondary effects in regards to increasing insulin secretion and suppressing lipolysis (40), suggesting that increased fasting EGP may be an adaptation that shifts whole-body fuel selection towards glucose in response to dietary carbohydrate surplus. Indeed, another previous study utilizing high-carbohydrate overfeeding (2) observed an increase in fasting insulin, as in the current study, but no change to insulin sensitivity as determined during the clamp, despite an increase in insulin signalling. This suggests that early adaptations in response to carbohydrate overfeeding are directed at increasing glucose disposal in order to maintain whole-body insulin sensitivity. Thus, in the current study, alterations to fasting EGP, Rd and insulin after 5 days of overfeeding likely represent a physiological adaptation to short-term energy excess to support a shift in whole-body metabolism toward increased carbohydrate oxidation.

In conclusion, the overfeeding model used in this study is likely more indicative of the human condition that leads weight gain, as opposed to the high-fat overfeeding models which produce larger effect sizes. Certainly the short-term (i.e. 5 d) overfeeding model is indicative of humans overeating during festivals and holidays and demonstrates that the regulatory system adapts to shift fuel usage towards glucose by increasing fasting EGP, plasma insulin and Rd, at least in young lean males who may be more metabolically flexible and hence

415 better able to buffer such nutritional oversupply. However, in response to chronic (i.e. 28 d)
416 overfeeding that results in small yet significant changes to body composition, the increases in
417 postprandial glycemia and insulinemia were modest, as the overfeeding macronutrient
418 composition was representative of participants' habitual diet. Indeed, it appears that the
419 modest increases in glycemia following chronic overfeeding with a typical 'Western' diet
420 occurs in order to maintain rates of glucose Rd and maintain suppression of EGP. Indeed, in
421 contrast to our hypothesis that defects in EGP suppression would occur rapidly in response to
422 overfeeding, suppression of EGP was maintained at both 5 and 28 days of overfeeding. Thus,
423 small increases in glycemia which can be considered to be within the 'normal healthy' range;
424 along with reduced insulin clearance, may work to minimize the burden on beta cell insulin
425 secretion following periods of overfeeding.

426 Acknowledgements

427 G.M.K is supported by an Australian Research Council Discovery Early Career Research
428 Award (DE180100859). C.R.B is supported by an Australian Research Council Future
429 Fellowship (FT160100017). D.J.M. is the guarantor of this work and, as such, had full access
430 to all the data in the study and takes responsibility for the integrity of the data and the
431 accuracy of the data analysis. D.J.M., G.D.W., G.M.K. and C.R.B. performed experiments,
432 were involved in the data analysis and drafting of the manuscript. All authors critically
433 revised and approved the manuscript. D.J.M., G.D.W., G.M.K. and C.R.B have no conflicts
434 of interest to declare.

References

1. **(CDC) CfDCaP**. National Health and Nutrition Examination Survey Data https://www.cdc.gov/nchs/data/hus/hus16.pdf#056: 2016.
2. **Adochio RL, Leitner JW, Gray K, Draznin B, and Cornier M-A**. Early responses of insulin signaling to high-carbohydrate and high-fat overfeeding. *Nutrition & Metabolism* 6: 37-37, 2009.
3. **Antoniewicz MR, Kelleher JK, and Stephanopoulos G**. Measuring deuterium enrichment of glucose hydrogen atoms by gas chromatography/mass spectrometry. *Analytical chemistry* 83: 3211-3216, 2011.
4. **Basu R, Di Camillo B, Toffolo G, Basu A, Shah P, Vella A, Rizza R, and Cobelli C**. Use of a novel triple-tracer approach to assess postprandial glucose metabolism. *American Journal of Physiology-Endocrinology And Metabolism* 284: E55-E69, 2003.
5. **Bergman RN, Phillips LS, and Cobelli C**. Physiologic evaluation of factors controlling glucose tolerance in man: measurement of insulin sensitivity and beta-cell glucose sensitivity from the response to intravenous glucose. *Journal of Clinical Investigation* 68: 1456-1467, 1981.
6. **Best JD, Kahn SE, Ader M, Watanabe RM, Ni TC, and Bergman RN**. Role of glucose effectiveness in the determination of glucose tolerance. *Diabetes Care* 19: 1018-1030, 1996.
7. **Bock G, Dalla Man C, Campioni M, Chittilapilly E, Basu R, Toffolo G, Cobelli C, and Rizza R**. Pathogenesis of pre-diabetes: mechanisms of fasting and postprandial hyperglycemia in people with impaired fasting glucose and/or impaired glucose tolerance. *Diabetes* 55: 3536-3549, 2006.
8. **Bonnard C, Durand A, Peyrol S, Chanseaume E, Chauvin M-A, Morio B, Vidal H, and Rieusset J**. Mitochondrial dysfunction results from oxidative stress in the skeletal muscle of diet-induced insulin-resistant mice. *Journal of Clinical Investigation* 118: 789-800, 2008.
9. **Bonnet F, Ducluzeau P-H, Gastaldelli A, Laville M, Anderwald CH, Konrad T, Mari A, Balkau B, and Group RS**. Liver enzymes are associated with hepatic insulin resistance, insulin secretion, and glucagon concentration in healthy men and women. *Diabetes* 60: 1660-1667, 2011.
10. **Brøns C, Jensen CB, Storgaard H, Hiscock NJ, White A, Appel JS, Jacobsen S, Nilsson E, Larsen CM, and Astrup A**. Impact of short-term high-fat feeding on glucose and insulin metabolism in young healthy men. *The Journal of physiology* 587: 2387-2397, 2009.
11. **Browning JD, and Horton JD**. Molecular mediators of hepatic steatosis and liver injury. *Journal of Clinical Investigation* 114: 147, 2004.
12. **Chen M, Liu B, Thompson CH, Wittert GA, and Heilbronn LK**. Acute Overfeeding Does Not Alter Liver or Adipose Tissue-Derived Cytokines in Healthy Humans. *Annals of nutrition & metabolism* 69: 165-170, 2016.
13. **Clore JN, Helm ST, and Blackard WG**. Loss of hepatic autoregulation after carbohydrate overfeeding in normal man. *Journal of Clinical Investigation* 96: 1967-1972, 1995.
14. **Cornford AS, Hinko A, Nelson RK, Barkan AL, and Horowitz JF**. Rapid development of systemic insulin resistance with overeating is not accompanied by robust changes in skeletal muscle glucose and lipid metabolism. *Applied Physiology, Nutrition, and Metabolism* 38: 512-519, 2012.
15. **Cornier M-A, Bergman BC, and Bessesen DH**. The effects of short-term overfeeding on insulin action in lean and reduced-obese individuals. *Metabolism* 55: 1207-1214, 2006.
16. **Frank JW, Saslow SB, Camilleri M, Thomforde GM, Dinneen S, and Rizza RA**. Mechanism of accelerated gastric emptying of liquids and hyperglycemia in patients with type II diabetes mellitus. *Gastroenterology* 109: 755-765, 1995.
17. **Gastaldelli A, Cusi K, Pettiti M, Hardies J, Miyazaki Y, Berria R, Buzzigoli E, Sironi AM, Cersosimo E, Ferrannini E, and Defronzo RA**. Relationship between hepatic/visceral fat and hepatic insulin resistance in nondiabetic and type 2 diabetic subjects. *Gastroenterology* 133: 496-506, 2007.
18. **Guariguata L, Whiting D, Hambleton I, Beagley J, Linnenkamp U, and Shaw J**. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes research and clinical practice* 103: 137-149, 2014.
19. **Horton TJ, Drougas H, Brachey A, Reed GW, Peters JC, and Hill JO**. Fat and carbohydrate overfeeding in humans: different effects on energy storage. *Am J Clin Nutr* 62: 19-29, 1995.

20. **Hovorka R, Soons PA, and Young MA**. ISEC: a program to calculate insulin secretion. *Computer methods and programs in biomedicine* 50: 253-264, 1996.
21. **Hu G, Lindstrom J, Valle TT, Eriksson JG, Jousilahti P, Silventoinen K, Qiao Q, and Tuomilehto J**. Physical activity, body mass index, and risk of type 2 diabetes in patients with normal or impaired glucose regulation. *Archives of internal medicine* 164: 892-896, 2004.
22. **Hulston CJ, Churnside AA, and Venables MC**. Probiotic supplementation prevents high-fat, overfeeding-induced insulin resistance in human subjects. *British Journal of Nutrition* 113: 596-602, 2015.
23. **Kahn SE, Prigeon RL, McCulloch DK, Boyko EJ, Bergman RN, Schwartz MW, Neifing JL, Ward WK, Beard JC, Palmer JP, and et al.** Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. *Diabetes* 42: 1663-1672, 1993.
24. **Kleemann R, van Erk M, Verschuren L, van den Hoek AM, Koek M, Wielinga PY, Jie A, Pellis L, Bobeldijk-Pastorova I, and Kelder T**. Time-resolved and tissue-specific systems analysis of the pathogenesis of insulin resistance. *PloS one* 5: e8817, 2010.
25. **Knudsen SH, Hansen LS, Pedersen M, Dejgaard T, Hansen J, Van Hall G, Thomsen C, Solomon TP, Pedersen BK, and Krogh-Madsen R**. Changes in insulin sensitivity precede changes in body composition during 14 days of step reduction combined with overfeeding in healthy young men. *Journal of applied physiology* 113: 7-15, 2012.
26. **Kowalski GM, and Bruce CR**. The regulation of glucose metabolism: implications and considerations for the assessment of glucose homeostasis in rodents. *American Journal of Physiology-Endocrinology and Metabolism* 307: E859-E871, 2014.
27. **Kraegen EW, Clark PW, Jenkins AB, Daley EA, Chisholm DJ, and Storlien LH**. Development of muscle insulin resistance after liver insulin resistance in high-fat–fed rats. *Diabetes* 40: 1397-1403, 1991.
28. **Lagerpusch M, Bosy-Westphal A, Kehden B, Peters A, and Müller M**. Effects of brief perturbations in energy balance on indices of glucose homeostasis in healthy lean men. *International journal of obesity* 36: 1094-1101, 2011.
29. **Lammert O, Grunnet N, Faber P, Bjørnsbo KS, Dich J, Larsen LO, Neese RA, Hellerstein MK, and Quistorff B**. Effects of isoenergetic overfeeding of either carbohydrate or fat in young men. *British Journal of Nutrition* 84: 233-245, 2000.
30. **Lee W, Bassilian S, Guo Z, Schoeller D, Edmond J, Bergner E, and Byerley L**. Measurement of fractional lipid synthesis using deuterated water (2H2O) and mass isotopomer analysis. *American Journal of Physiology-Endocrinology And Metabolism* 266: E372-E383, 1994.
31. **Mason SA, Della Gatta PA, Snow RJ, Russell AP, and Wadley GD**. Ascorbic acid supplementation improves skeletal muscle oxidative stress and insulin sensitivity in people with type 2 diabetes: Findings of a randomized controlled study. *Free radical biology & medicine* 93: 227-238, 2016.
32. **Morrison DJ, Kowalski GM, Grespan E, Mari A, Bruce CR, and Wadley GD**. Measurement of postprandial glucose fluxes in response to acute and chronic endurance exercise in healthy humans. *American Journal of Physiology-Endocrinology and Metabolism* 2017.
33. **Park S-Y, Cho Y-R, Kim H-J, Higashimori T, Danton C, Lee M-K, Dey A, Rothermel B, Kim Y-B, and Kalinowski A**. Unraveling the temporal pattern of diet-induced insulin resistance in individual organs and cardiac dysfunction in C57BL/6 mice. *Diabetes* 54: 3530-3540, 2005.
34. **Parry SA, Smith JR, Corbett TR, Woods RM, and Hulston CJ**. Short-term, high-fat overfeeding impairs glycaemic control but does not alter gut hormone responses to a mixed meal tolerance test in healthy, normal-weight individuals. *British Journal of Nutrition* 117: 48-55, 2017.
35. **Peterson C, Zhang B, Johannsen D, and Ravussin E**. Eight weeks of overfeeding alters substrate partitioning without affecting metabolic flexibility in men. *International Journal of Obesity* 41: 887-893, 2017.

36. **Pietrobelli A, Formica C, Wang Z, and Heymsfield SB**. Dual-energy X-ray absorptiometry body composition model: review of physical concepts. *American Journal of Physiology-Endocrinology And Metabolism* 271: E941-E951, 1996.
37. **Polonsky KS, Gumbiner B, Ostrega D, Griver K, Tager H, and Henry RR**. Alterations in immunoreactive proinsulin and insulin clearance induced by weight loss in NIDDM. *Diabetes* 43: 871-877, 1994.
38. **Samocha-Bonet D, Campbell L, Viardot A, Freund J, Tam CS, Greenfield JR, and Heilbronn LK**. A family history of type 2 diabetes increases risk factors associated with overfeeding. *Diabetologia* 53: 1700-1708, 2010.
39. **Samocha-Bonet D, Campbell LV, Mori TA, Croft KD, Greenfield JR, Turner N, and Heilbronn LK**. Overfeeding reduces insulin sensitivity and increases oxidative stress, without altering markers of mitochondrial content and function in humans. *PloS one* 7: e36320, 2012.
40. **Schwarz J-M, Neese RA, Turner S, Dare D, and Hellerstein MK**. Short-term alterations in carbohydrate energy intake in humans. Striking effects on hepatic glucose production, de novo lipogenesis, lipolysis, and whole-body fuel selection. *Journal of Clinical Investigation* 96: 2735, 1995.
41. **Smith GI, Magkos F, Reeds DN, Okunade AL, Patterson BW, and Mittendorfer B**. One Day of Mixed Meal Overfeeding Reduces Hepatic Insulin Sensitivity and Increases VLDL Particle But Not VLDL-Triglyceride Secretion in Overweight and Obese Men. *The Journal of Clinical Endocrinology and Metabolism* 98: 3454-3462, 2013.
42. **Statistics ABo**. Australian Health Survey: Nutrition First Results—Foods and Nutrients http://www.abs.gov.au/ausstats/abs@.nsf/Lookup/by%20Subject/4364.0.55.007~2011-12~Main%20Features~Key%20Findings~1: 2011–12.
43. **Steele R, Wall J, De Bodo R, and Altszuler N**. Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *American Journal of Physiology--Legacy Content* 187: 15-24, 1956.
44. **Thyfault JP, and Krogh-Madsen R**. Metabolic disruptions induced by reduced ambulatory activity in free-living humans. *Journal of applied physiology (Bethesda, Md : 1985)* 111: 1218-1224, 2011.
45. **Toffolo G, Dalla Man C, Cobelli C, and Sunehag AL**. Glucose fluxes during OGTT in adolescents assessed by a stable isotope triple tracer method. *Journal of Pediatric Endocrinology and Metabolism* 21: 31-46, 2008.
46. **Turner N, Kowalski G, Leslie S, Risis S, Yang C, Lee-Young R, Babb J, Meikle P, Lancaster G, and Henstridge D**. Distinct patterns of tissue-specific lipid accumulation during the induction of insulin resistance in mice by high-fat feeding. *Diabetologia* 56: 1638-1648, 2013.
47. **Usui C, Asaka M, Kawano H, Aoyama T, Ishijima T, Sakamoto S, and Higuchi M**. Visceral fat is a strong predictor of insulin resistance regardless of cardiorespiratory fitness in non-diabetic people. *Journal of nutritional science and vitaminology* 56: 109-116, 2010.
48. **Vergnaud A-C, Norat T, Mouw T, Romaguera D, May AM, Bueno-de-Mesquita HB, van der A D, Agudo A, Wareham N, Khaw K-T, Romieu I, Freisling H, Slimani N, Perquier F, Boutron-Ruault M-C, Clavel-Chapelon F, Palli D, Berrino F, Mattiello A, Tumino R, Ricceri F, Rodríguez L, Molina-Montes E, Amiano P, Barricarte A, Chirlaque M-D, Crowe FL, Orfanos P, Naska A, Trichopoulou A, Teucher B, Kaaks R, Boeing H, Buijsse B, Johansson I, Hallmans G, Drake I, Sonestedt E, Jakobsen MU, Overvad K, Tjønneland A, Halkjær J, Skeie G, Braaten T, Lund E, Riboli E, and Peeters PHM**. Macronutrient Composition of the Diet and Prospective Weight Change in Participants of the EPIC-PANACEA Study. *PLoS ONE* 8: e57300, 2013.
49. **Wadden TA, Brownell KD, and Foster GD**. Obesity: responding to the global epidemic. *Journal of consulting and clinical psychology* 70: 510, 2002.

Table 1: Anthropometric and dietary data at baseline, after short-term (5 days) and chronic (28 days) of overfeeding.

Characteristic	Baseline	Short-term Overfeeding (5 days)	Chronic Overfeeding (28 days)
Age, years	22.8 ± 0.3	_____	_____
Height, cm	179.0 ± 0.2	_____	_____
Mass, kg	79.96 ± 0.80	80.65 ± 0.77	81.60 ± 0.77 *
BMI, $kg.m^{-2}$	24.65 ± 0.30	24.89 ± 0.28	25.18 ± 0.28 *
Lean Mass, kg	59.11 ± 0.58	60.21 ± 0.53	59.91 ± 0.53
Fat Mass, kg	17.59 ± 0.81	17.68 ± 0.80	18.90 ± 0.79 **
Visceral Fat Volume, $g.cm^{-2}$	434.38 ± 8.74	493.88 ± 8.22 **	504.5 ± 9.03 *
Dietary Energy, kj	11211 ± 353	16053 ± 629 **	16318 ± 542 **
Fat, % total energy	32.5 ± 1.8	34.0 ± 2.2	31.0 ± 1.9
Fat, g/day	98.2 ± 5.3	148.3 ± 11.3 **	137.4 ± 8.9 **
Carbohydrate, % total energy	45.2 ± 0.6	44.8 ± 1.3	48.6 ±2.2
Carbohydrate, g/day	298.6 ± 12.8	421.7 ± 16.9 **	465.6 ± 14.5 **
Protein, % total energy	18.1 ± 1.0	16.6 ± 1.3	16.7 ± 1.4
Protein, g/day	118.9 ± 6.9	155.7 ± 9.2 **	158.9 ± 9.9 **

585

586 *Values are in Mean ± SEM, n = 8. N/A = not applicable. * = P<0.05;** = P<0.01 versus*

587 *baseline, as determined by paired t-test.*

588 Table 2: The effect of short-term (5 days) and chronic (28 days) of overfeeding on fasting and

589 postprandial plasma hormones and metabolites.

Concentrations	Baseline	Short-term Overfeeding (5 days)	Chronic Overfeeding (28 days)
Fasting Glucose, $mmol.l^{-1}$	4.42 ± 0.15	4.55 ± 0.13	4.58 ± 0.13
Glucose AUC, $mmol.l^{-1}$ x min	1279.0 ± 99.4	1203.1 ± 80.0	1398.2 ± 80.7
Glucose 0-120min AUC, $mmol.l^{-1}$ x min	647.0 ± 53.7	618.1 ± 45.9	736.3 ± 46.5 *
Fasting Insulin, $pmol.l^{-1}$	25.91 ± 8.06	30.29 ± 7.75 *	27.33 ± 6.67
Insulin AUC, $pmol.l^{-1}$ x min	53812 ± 11069	62315 ± 13545	71107 ± 16982
Insulin 0-120min AUC, $pmol.l^{-1}$ x min	39487 ± 7923	49754 ± 11659	51710 ± 11927 *
Fasting C-peptide, $nmol.l^{-1}$	0.33 ± 0.05	0.35 ± 0.06	0.34 ± 0.05
C-Peptide AUC, $nmol.l^{-1}$ x min	298.5 ± 45.2	300.6 ± 40.9	330.5 ± 47.8
C-peptide 0-120min AUC, $nmol.l^{-1}$ x min	174.9 ± 24.1	186.4 ± 27.5	198.4 ± 30.8
Fasting TAG, $mmol.l^{-1}$	1.08 ± 0.08	1.03 ± 0.06	1.21 ± 0.16
TAG AUC, $mmol.l^{-1}$ x min	259.2 ± 25.2	229.5 ± 23.0	283.5 ± 36.2
TAG AUC 0-120min, $mmol.l^{-1}$ x min	125.1 ± 11.0	112.3 ± 10.6	141.2 ± 17.8
Fasting NEFA, $mmol.l^{-1}$	0.19 ± 0.03	0.15 ± 0.03	0.16 ± 0.03
NEFA AUC, $mmol.l^{-1}$ x min	25.4 ± 4.1	23.1 ± 4.0	25.5 ± 3.8
NEFA AUC 0-120min, $mmol.l^{-1}$ x min	11.0 ± 1.7	9.3 ± 1.6	10.9 ± 1.4

590

591 AUC, area under the curve; TAG, triglycerides; NEFA, non-esterified fatty acids. *Values are*

592 *in Mean ± SEM, n = 8. * P<0.05 versus baseline, as determined by paired t-test.*

Figure 1: Plasma glucose concentration (A), plasma insulin concentration (B), plasma C-peptide concentration (C), insulin secretion rate (D), and plasma non-esterified fatty acids (NEFA) (E); in healthy young men during a 4.5h labelled mixed meal tolerance test; at baseline and after short-term overfeeding (5 days). *Plots are mean ± SEM, n=8. * P<0.05, ** P<0.01, *** P<0.001 vs. baseline, as determined by Bonferonni post-hoc analysis.*

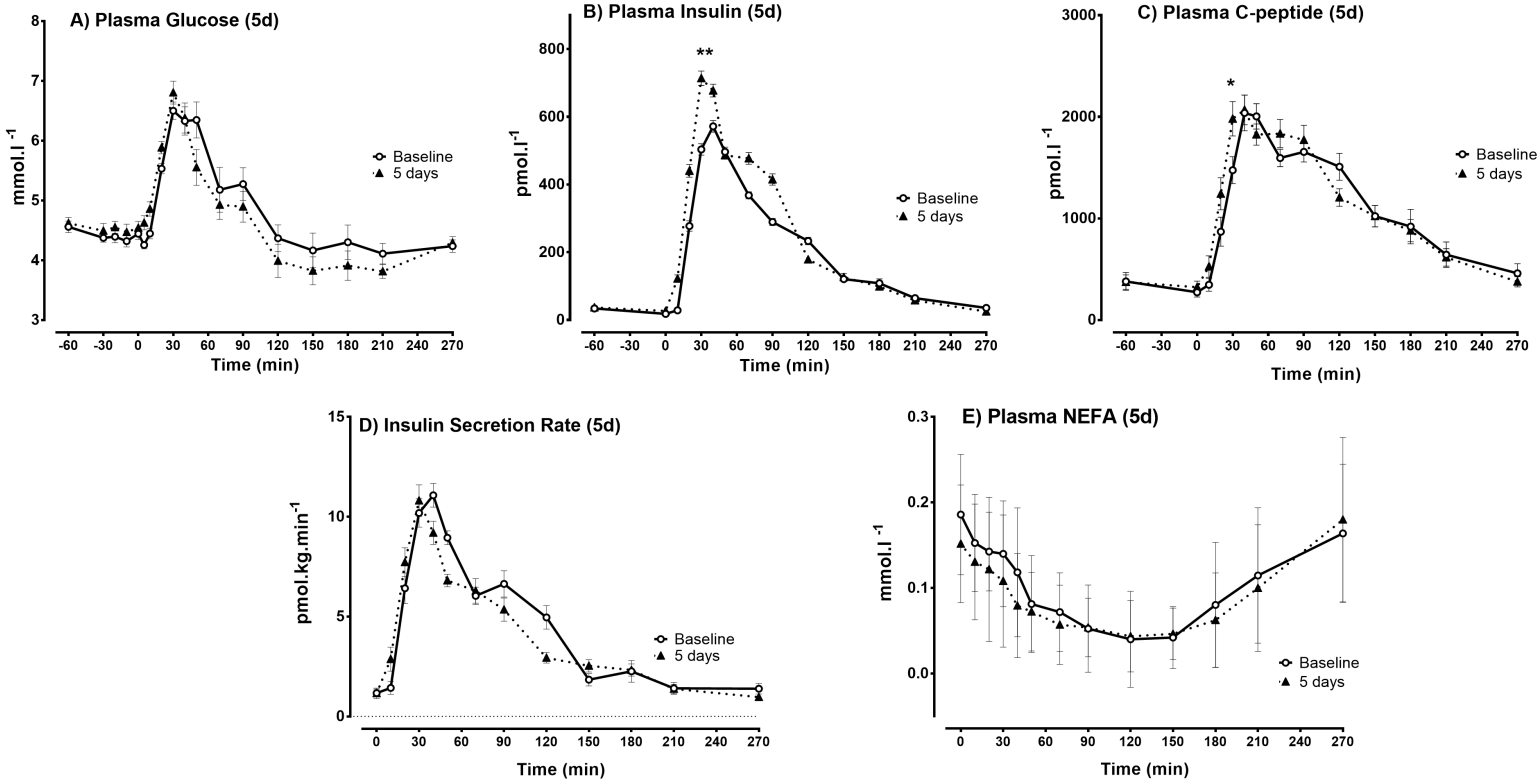
Figure 2: Plasma glucose concentration (A), plasma insulin concentration (B), plasma C-peptide concentration (C), insulin secretion rate (D), and plasma non-esterified fatty acids (NEFA) (E); in healthy young men during a 4.5h labelled mixed meal tolerance test; at baseline and after chronic overfeeding (28 days). *Plots are mean ± SEM, n=8. * P<0.05, ** P<0.01, *** P<0.001 vs. baseline, as determined by Bonferonni post-hoc analysis.*

Figure3: Tracer-to-tracee ratios for [1-^{13}C] glucose to endogenous glucose (A), and [U-^{13}C] glucose to [6,6 – ^{2}H] glucose (B) in healthy young men; at baseline, after short-term overfeeding (5 days) and after chronic overfeeding (28 days). *Plots are mean ± SEM, n=8.*

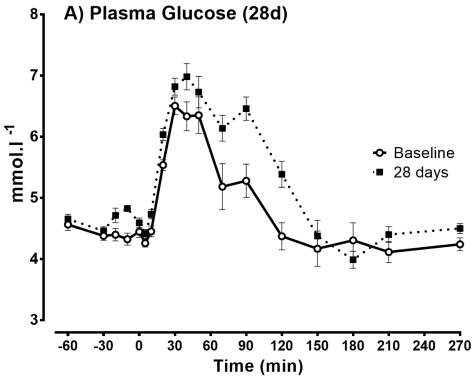
Figure 4: Meal glucose rate of appearance (A; D), glucose rate of disposal (B; E), and endogenous glucose production (C; F) in healthy young men during a 4.5h labelled mixed meal tolerance test; at baseline, after short-term overfeeding (5 days) and after chronic overfeeding (28 days). *Plots are mean ± SEM, n=8. # P<0.05 vs baseline, as determined by one-way ANOVA; * P<0.05, ** P<0.01, *** P<0.001 vs. baseline, as determined by Bonferroni post-hoc analysis.*

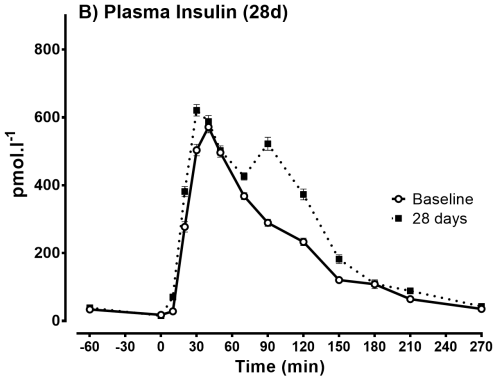
Figure 5: Plasma meal glucose concentration (A; C) and endogenous glucose concentration (B; D) in in healthy young men during a 4.5h labelled mixed meal tolerance test; at baseline, after short-term overfeeding (5 days) and after chronic overfeeding (28 days). *Plots are mean ± SEM, n=8. * P<0.05, ** P<0.01, *** P<0.001 vs. baseline as determined by Bonferroni post-hoc analysis.*

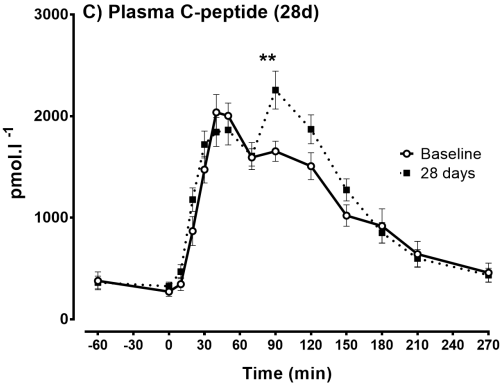
5 days overfeeding

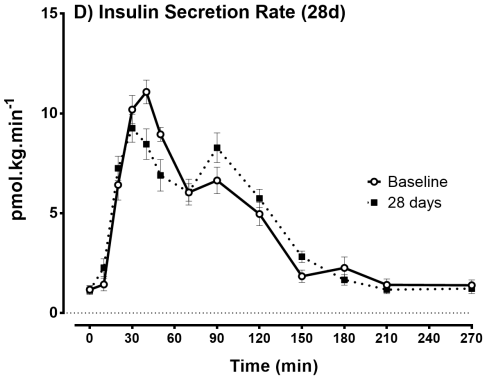


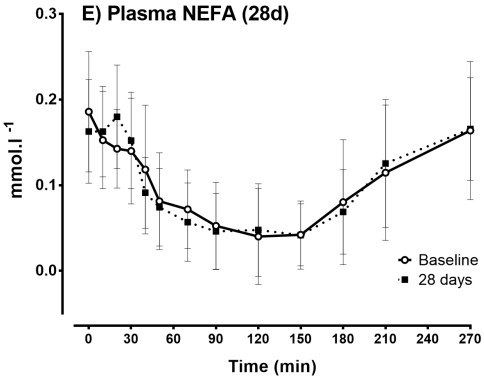
28 days overfeeding

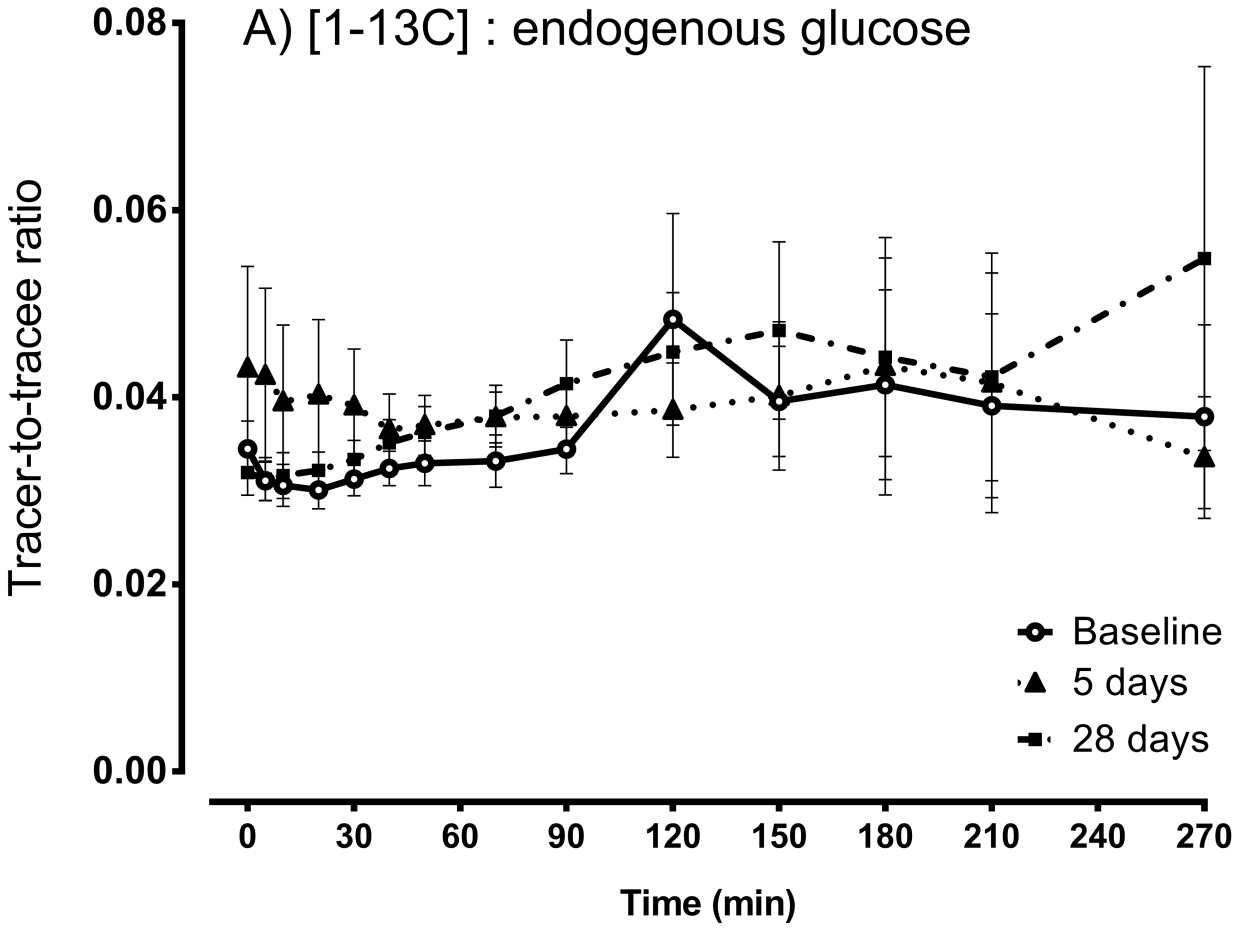


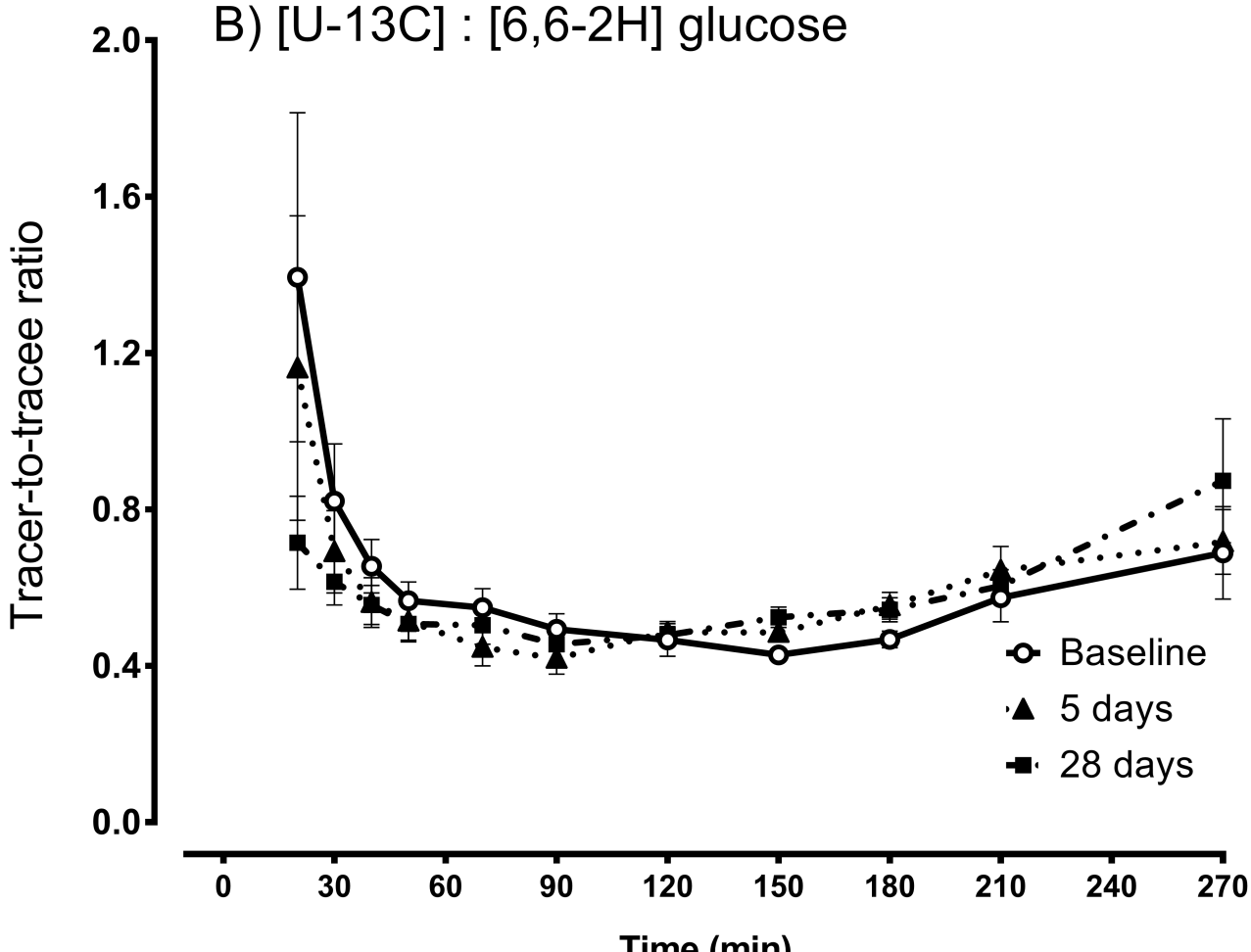




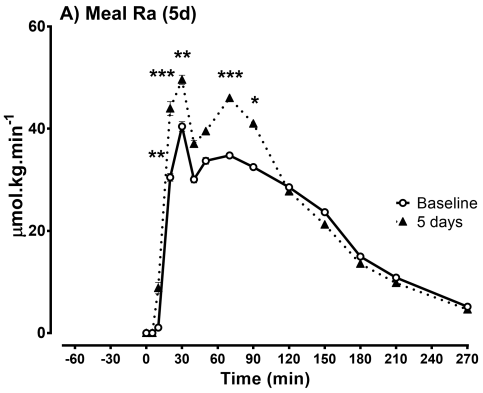


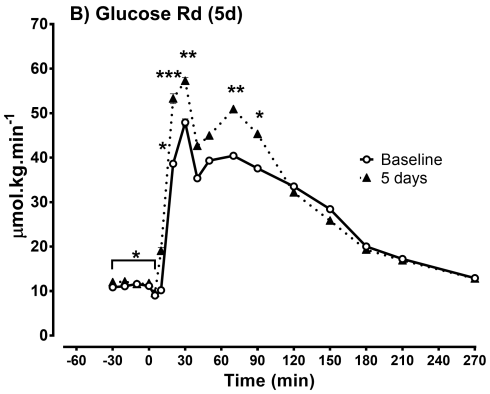


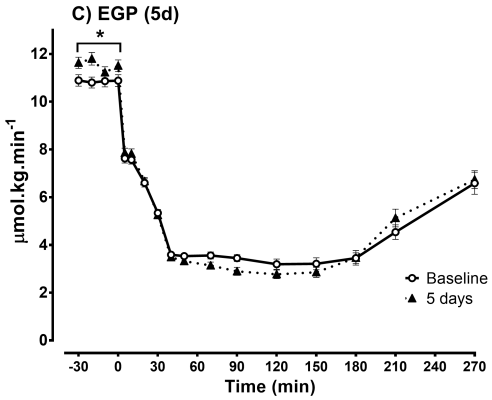




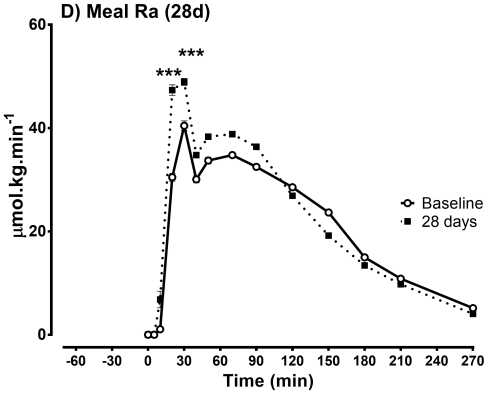
5 days overfeeding

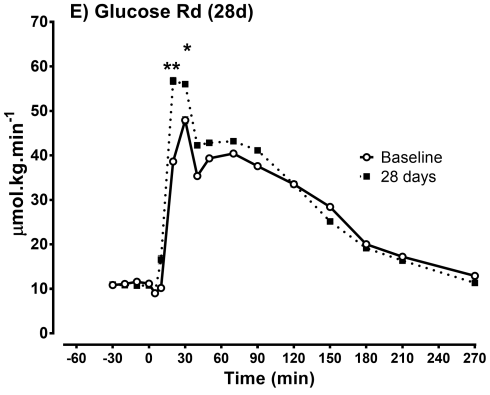


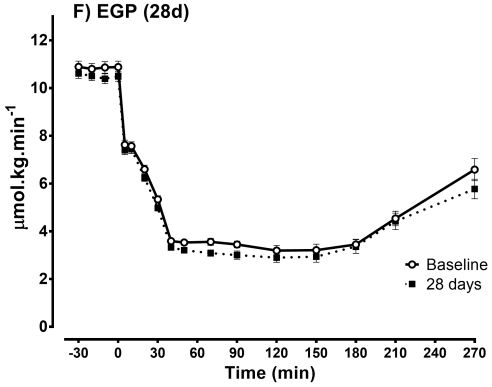




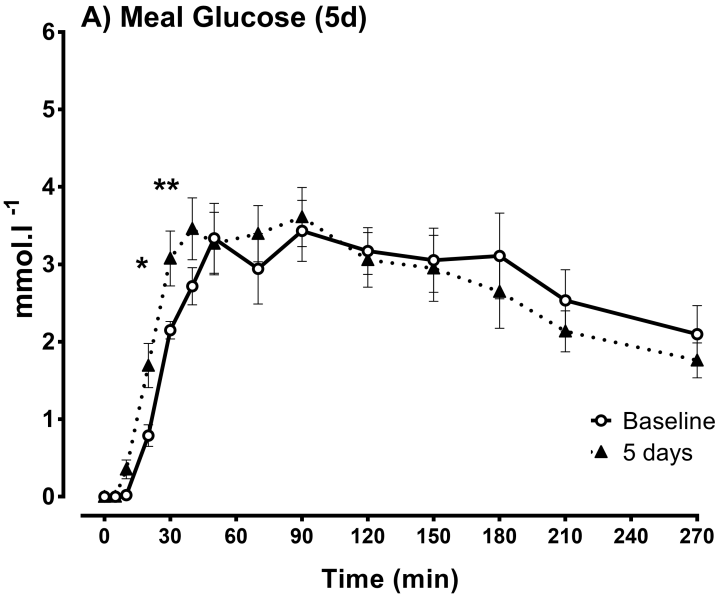
28 days overfeeding

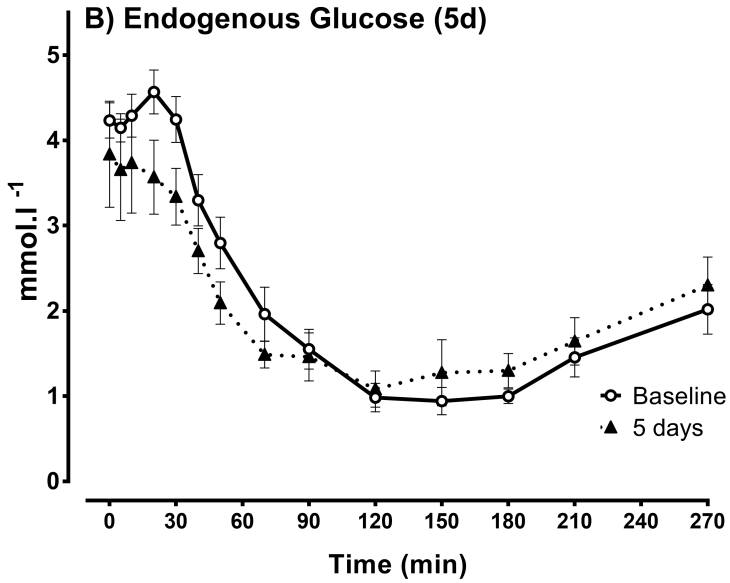






5 days overfeeding





28 days overfeeding