What have human experimental overfeeding studies taught us about adipose tissue expansion and susceptibility to obesity and metabolic complications?

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Abstract

Overfeeding experiments, in which we impose short-term positive energy balance, help unravel the cellular, physiological and behavioural adaptations to nutrient excess. These studies mimic longer-term mismatched energy expenditure and intake. There is considerable inter-individual heterogeneity in the magnitude of weight gain when exposed to similar relative caloric excess reflecting variable activation of compensatory adaptive mechanisms. Significantly, given similar relative weight gain, individuals maybe protected from/predisposed to metabolic complications (insulin resistance, dyslipidaemia, hypertension), non-alcoholic fatty liver disease and cardiovascular disease. Similar mechanistic considerations underpinning the heterogeneity of overfeeding responses are pertinent in understanding emerging metabolic phenotypes e.g. metabolically unhealthy normal weight and metabolically healthy obesity.

Intrinsic and extrinsic factors modulate individuals' overfeeding response: intrinsic factors include genetic/ethnic background, baseline metabolic health and regional fat distribution; extrinsic factors include macronutrient (fat *vs.* carbohydrate) content, fat/carbohydrate composition and overfeeding pattern (larger portions *vs*. snacks).

Subcutaneous adipose tissue (SAT) analysis, coupled with metabolic assessment, with overfeeding have revealed how SAT remodels to accommodate excess nutrients. Healthy remodeling involves adipocyte *hyperplasia;* dysfunctional remodeling involves *hypertrophy* inducing inflammation and insulin resistance. Biological responses of SAT also govern the extent of ectopic (visceral/liver) fat deposition. Body composition analysis by DEXA/MRI have determined the relative expansion of SAT (including abdominal/gluteofemoral SAT) *versus* ectopic fat with overfeeding.

Such studies have contributed to the *adipose expandability hypothesis* whereby SAT

has a finite capacity to expand (governed by intrinsic biological characteristics) and once capacity is exceeded ectopic fat deposition occurs. The potential for SAT expandability confers protection from/predisposes to the adverse metabolic responses to over-feeding. The concept of a *personal fat threshold* suggests a large inter-individual variation in SAT capacity with ectopic fat/metabolic decompensation once one's own threshold is exceeded.

This review summarises insight gained from overfeeding studies regarding susceptibility to obesity and related complications with nutrient excess.

Introduction

Long-term regulation and maintenance of body weight and body composition relies upon integrated systems controlling energy intake, energy expenditure, substrate utilisation and partitioning among different metabolic tissues and pathways. Peripheral signals released from the gastrointestinal tract and adipose tissue integrate within the hypothalamus to regulate energy intake and energy expenditure. Fat-free mass, through the resting metabolic rate, also regulates energy intake. It has been proposed that body weight is maintained at a 'set-point' and that deviations from this point (with negative or positive energy balance) are countered and minimised by feedback mechanisms involving compensatory changes in appetite and energy expenditure[1, 2].

Obesity represents a state of energy imbalance created by mismatched energy expenditure (reduced physical activity) and energy intake (nutrient excess). However, individuals subjected to a similar relative positive energy balance show considerable heterogeneity in the extent to which their body weight or body composition is altered. Fat has the greatest storage capacity of the macronutrients; protein and carbohydrate

have a much lesser capacity. Thus, body weight change occurs predominantly via alterations in adipose tissue volume with a much smaller contribution from changes in lean body mass.

There is abundant information on weight loss (achieved in many different ways) but much less information on controlled weight gain. Overfeeding experiments, in which we mimic a short-term state of energy imbalance, have facilitated our understanding of the adaptive cellular, physiological and behavioral responses of adipose tissue and other organs (e.g. liver, skeletal muscle and brain) to weight gain and helped explain the inter-individual heterogeneity to weight gain. These studies have also provided insight into susceptibility to metabolic decompensation with weight gain.

This is a narrative review, however, to ensure all relevant literature is considered, systematic searches were carried out on Medline and Scopus using the terms "overfeeding", "overeating", "hypercaloric", "controlled weight gain" and "experimental weight gain" limited to English language papers with human subjects. 2272 abstracts were screened, with 168 articles reporting the effects of hypercaloric diets in humans identified. This was supplemented by manual searches of reference lists. Reports from important overfeeding studies are described in this review, with data from experimental studies addressing the different baseline participant characteristics, overfeeding regimes imposed and imaging techniques (Table 1), effects on adipose tissue and ectopic fat distribution, adipocyte and metabolic responses (Table 2) and on adipokines, gut hormones and appetite regulation (Table 3).

Lessons learnt from early overfeeding studies

Forty years ago, to understand the biological response of adipose tissue to weight gain (hyperplasia *vs.* hypertrophy), Sims *et al* conducted a landmark overfeeding study in inmates at Vermont State Prison[3]. He studied 5 lean individuals, with no family history of obesity, and in exchange for early parole subjected them to 10 weeks of supervised overfeeding while they remained sedentary. They were fed a diet of their own choice consisting of a three-fold higher caloric intake than would be needed to maintain body weight, aiming for 15-25% weight gain.

Underlying the significant mean weight gain was a considerable inter-individual weight change between the inmates. The findings highlighted that the magnitude of weight gain cannot be predicted from the magnitude of positive calorie balance, with some individuals protected from, or predisposed to, weight gain through a variety of mechanisms. The key finding was that fat mass expansion occurred via an increase in adipocyte cell size rather than cell number *i.e.* adipocyte hypertrophy rather than hyperplasia occurred.

Genetic basis for fat distribution and metabolic health

Body fat distribution appears intrinsic to the individual and is likely to depend on heritable factors such as genetic variants, which are likely also subject to epigenetic regulation. A recent study identified 49 genetic loci associated with waist-to-hip ratio (adjusted for BMI), showing a stronger effect in women. These loci were enriched for genes expressed in adipose tissue with pathway analysis implicating adipogenesis, angiogenesis and insulin resistance as processes influencing fat distribution[4].

Several recent publications have highlighted several specific (common) genetic variants (particularly those associated with insulin resistance) where there is dissociation between the body mass index (BMI) and the risk of type 2 diabetes

mellitus (T2DM) or cardiovascular disease (CVD) based on differing body composition/regional fat distribution[5, 6]. Genetic evidence has been provided for normal weight/lower BMI individuals with a metabolically obese phenotype, incorporating components of the metabolic syndrome and whose body composition is characterised by greater hepatic steatosis and increased visceral adipose tissue (VAT) relative to subcutaneous adipose tissue (SAT) (i.e. lower SAT capacity). These individuals were at an increased risk of T2DM, coronary artery disease or hypertension[5]. Conversely, genetic evidence has been provided for individuals with a higher BMI but lower risk of T2DM, hypertension and CVD. Presence of these 'favourable adiposity alleles' are associated with lower insulin levels and a higher SAT:VAT ratio (i.e. higher SAT capacity) [6].

The same genetic/epigenetic factors will also determine the pattern/distribution of fat depot expansion during weight gain.

Conceptual framework for fate of excess energy (Figure 1)

With overfeeding, there are two fates for the surplus energy: either through stimulation of energy expenditure or deposition in a storage depot **(Figure 1A)**. However, the majority of excess energy is stored, rather than expended; the amount stored representing the difference between total energy expended and total energy ingested. The surplus energy maybe stored in adipose tissue **(Figure 1B)** or as lean body mass **(Figure 1C)**. The biological properties of adipose tissue, and its response to overfeeding, profoundly influence the distribution of body fat change: upper *vs.* lower body fat *and* subcutaneous adipose tissue (SAT) *vs*. ectopic fat deposition including as visceral adipose tissue (VAT) or liver fat **(Figure 1D)**. The distribution

of excess body fat (whether stored as SAT, upper or lower body or as ectopic fat) has potentially profound secondary consequences on metabolic and cardiovascular risk.

Changes in energy expenditure with overfeeding (Figure 1A)

Total energy expenditure (TEE) is composed of resting energy expenditure (REE) (~60% of total), thermic effects of food and activity energy expenditure (exercise and non-exercise activity thermogenesis [7]).

TEE TEE is stimulated with overfeeding (by ~10%)[8] but does not increase linearly with weight gain[9]. The extent of TEE stimulation during overfeeding governs the amount of excess energy stored and thus associated weight gain: individuals with a lesser tendency to gain weight increase TEE to a greater extent. With ensuing weight gain, resting metabolic rate will further increase (related to increased body mass) with recalibration dependent upon the relative changes in fat volume *vs*. muscle mass (skeletal muscle has higher relative energy requirements relative to adipose tissue)[10].

The stimulation of REE also depends upon the macronutrient content of the overfeeding regime with a hierarchy of macronutrient oxidation; macronutrients with limited storage capacity are oxidized first. Fat overfeeding has minimal effect on fat oxidation and total energy expenditure, such that 90-95% of excess energy is stored, resulting in greater fat accumulation. In response to carbohydrate overfeeding, there is stimulation of carbohydrate oxidation and an increase in TEE with a lower proportion (75-85%) of energy stored [2]. Prolonged overfeeding carbohydrate increases body fat by stimulation of *de novo lipogenesis* of hepatic and extra-hepatic (adipose tissue) origin. The predominant effect of protein overfeeding is accretion of lean body mass with the effect of increasing resting metabolic rate[11].

Diet-induced thermogenesis (DIT) DIT, the energy expenditure associated with metabolising food, is also influenced by both the energy content and the macronutrient composition of the food ingested: isocaloric amounts of protein, carbohydrate and fat increase diet-induced energy expenditure by 20-30%, 5-10% and 0-3% of TEE respectively.

Activity energy expenditure (AEE) AEE is composed of energy expenditure related to spontaneous physical activity and non-exercise activity thermogenesis (NEAT). Differences in levels of NEAT have a greater impact on TEE than differences in spontaneous physical activity. Obese individuals tend to undertake less NEAT than lean individuals, being sedentary by a mean of 2 hours more per day[7]. NEAT has been shown to have a role in resistance to weight gain: individual susceptibility to overfeeding is determined by a variable induction in NEAT. 16 volunteers were overfed 1,000 calories daily for 2 months, with a mean weight gain of 10lb, but with a range of 2-16lb. Change in NEAT (kcal/day) was inversely correlated with fat gain (kg). Those with a high NEAT response were more protected from obesity with overfeeding; those with a low NEAT response were more susceptible to obesity with overfeeding[7].

Storage of excess energy (Figure 1B, C, D)

Weight gain during overfeeding cannot be oversimplified by assuming 3,500 calories equates to a 1lb/0.45kg change in body weight, even if the energy surplus during overfeeding is accurately quantified. This erroneous assumption is based upon the premise that body weight changes reflect primarily loss or gain of adipose tissue (comprising 87% triglyceride), knowing the energy density of fat to be 9 kcal/g. Longer term changes in body fat are accompanied by changes in lean tissue whose

metabolisable energy density is significantly less than body fat (4 kcal/g). Increased lean body mass would increase REE and higher body weight increases the energy requirement of physical activity. Mathematical models of energy expenditure and weight change have been developed that reflect the dynamic changes in body composition as weight increases[10].

A number of overfeeding studies have been performed with concomitant assessment of body composition by DEXA, CT and/or MRI to provide insight into which storage depot the excess energy is partitioned. Table 1 details the baseline participant characteristics and overfeeding regime used in overfeeding studies summarising those using concomitant assessment of body composition (*DEXA ± MRI*) to determine fate of excess energy into regional fat depots, with results summarized in Table 2.

Storage in adipose tissue vs. in lean body mass The concept of energy partitioning relates to the proportion of excess energy that is directed towards lean tissue *vs.* fat with the energy partition ratio being a non-linear function of body fat. People with a higher initial body fat have a greater fraction of their weight change attributable to increases in body fat *vs*. lean tissue[12].

Storage in upper body (abdominal) vs. lower body (gluteofemoral) fat. The regional distribution of SAT, quantified by DEXA, is critically important with subcutaneous fat depots in upper and lower body characterized by different structural and functional differences and therefore associated with different metabolic risk. Abdominal SAT (ASAT), i.e. upper body fat, is characterized by high uptake of diet-derived fat and a high lipid turnover. In contrast, gluteofemoral fat (GFAT) has a reduced lipid turnover but a high capacity to accommodate fat undergoing redistribution [13, 14].

Accumulation of adipose tissue in the upper body (abdominal obesity) is associated with increased risk of development of insulin resistance, type 2 diabetes mellitus and

higher cardiovascular and total mortality, independent of BMI. Indeed, individuals with a normal BMI and abdominal obesity (determined by waist-hip ratio) have a higher mortality compared with either individuals with a normal BMI without central obesity or with all overweight or obese individuals (based on BMI)[15]. Conversely, accumulation of fat in the lower body (gluteofemoral obesity) shows opposite associations with cardiovascular disease and type 2 diabetes mellitus when adjusted for overall fat mass. Paradoxically lower body fat accumulation is associated with improved cardiovascular and metabolic profiles (protective role) suggested to sequester lipids that would be destined for ectopic fat deposition[16].

Lower and upper body fat stores show a different response to weight gain reflecting their different biological characteristics and capacity for lipid storage/turnover[13].

Storage in subcutaneous adipose tissue vs. ectopic fat deposition (visceral adipose tissue and liver) Subcutaneous adipose tissue (SAT) must undergo expansion to accommodate increased lipid supply to avoid deposition of lipids/fatty acids in non-adipocyte cells (causing lipotoxicity)[17]. SAT expansion may occur by two distinct mechanisms: *hypertrophy* of existing adipocytes or promotion of differentiation of pre-adipocytes (*hyperplasia*).

The *adipose tissue expandability hypothesis* has suggested capacity for AT expansion is determined by functional adipocyte characteristics and their molecular and biochemical adaptive responses to positive energy balance[18]. This capacity is limited and determines the propensity for excess lipids to be orientated to other tissues *i.e.* ectopic lipid deposition, with secondary lipotoxicity. Taylor *et al.*, proposed a large inter-individual variation in the SAT buffering capacity with each individual having a *personal fat threshold*[19]. This means that once the SAT storage capacity is reached, ectopic fat deposition ensues with associated lipotoxicity and metabolic dysfunction.

These concepts of a finite AT expandability, which has large intra-individual variation, may explain the distinct body composition phenotypes of metabolic healthy and unhealthy, lean or obese[20]. Body composition analysis from these individuals have confirmed that metabolically unhealthy normal weight individuals are characterised by a low capacity for SAT expandability (*low personal fat threshold*) hence their higher lipid deposition in other organs (resulting in a higher VAT:SAT ratio and higher liver fat)[21]. Conversely, metabolically healthy obese individuals are characterised by a high capacity for SAT expandability (*high personal fat threshold*) (a lower VAT:SAT ratio and lower liver fat content)[20].

Insights from transgenic mice (lacking leptin while overexpressing adiponectin) demonstrate that massive expansion of SAT is metabolically inert, providing a safe harbor for potentially toxic lipids, with reduced ectopic fat (e.g. liver and visceral fat) and preserved insulin sensitivity with little/no systemic inflammation [22]. In contrast, a reduced capacity for SAT expansion is associated with subsequent inflammatory consequences, development of systemic insulin resistance (IR) and metabolic syndrome (MS), associated with subsequent development of endothelial dysfunction and atherosclerosis. These findings are borne out by observations in people with generalised lipodystrophy, who have limited capacity for subcutaneous fat storage and consequently develop severe insulin resistance, NAFLD and dyslipidaemia[23]. Conversely, the PPARϒ agonists thiazolidinediones improve metabolic profiles by promoting adipogenesis and increasing fat mass[24].

Healthy and dysfunctional adipose tissue remodeling and metabolic consequences

Healthy AT remodeling involves all cellular components of adipose tissue and not just adipocytes, with induction of various pathways within adipose tissue including that of lipid metabolism, the renin-angiotensin pathway, angiogenesis and extracellular matrix[25]. 'Healthy' SAT expansion consists of *hyperplasia*, AT enlargement through recruitment of adipogenic precursor cells, stimulation of angiogenesis and remodeling of the extracellular matrix (ECM); 'unhealthy' SAT expansion consists of adipocyte *hypertrophy* with limited angiogenesis and hypoxia resulting in secondary changes involving induction of tissue fibrosis[26], adipocyte cell death and enhanced pro-inflammatory cytokine secretion[27]. During this process there is a phenotypic switch with an infiltration of pro-inflammatory (M1) macrophages from the anti-inflammatory (M2) phenotype[28].

A number of overfeeding studies have tested the validity of the adipose tissue expandability hypothesis by concomitantly examining changes in adipose tissue (morphology, gene and protein expression), body composition (using DEXA and/or MRI/^{1}H-MRS) and the metabolic consequences (using oral glucose tolerance test or euglycaemic clamps) (summarised in Table 2). Thus we are able to simultaneously examine adaptations of the adipocytes structurally (e.g. adipocyte cell size, number and size distribution) and functionally (e.g. changes in expression of lipid metabolism genes) coupled with regional fat responses and partitioning of fat into different tissues (SAT *vs.* ectopic deposition). Such studies have provided mechanistic insight into how dysfunctional SAT remodeling contributes to visceral and liver fat deposition (clinically as non-alcoholic fatty liver disease, NAFLD) and in doing so initiating metabolic dysfunction with development of components of metabolic syndrome (dyslipidaemia, hypertension, insulin resistance).

Alligier *et al.* overfed participants an additional daily lipid mixture composed of 70g

(760 kcal) of saturated and monounsaturated fatty acids for 56 days[29]. Mean body weight change was 2.5 kg with substantial inter-individual heterogeneity in magnitude of weight gain and in the relative accretion of subcutaneous *vs.* visceral fat. Although the increment in SAT was associated with the increase in body weight, there was no relationship between the increment in body weight and VAT nor was there any association between the expansion of SAT and VAT volumes. The magnitude of the increase in VAT volume was positively correlated with the magnitude of the post-prandial exogenous fatty acid release in the circulation during a labelled palmitate test meal. Using SAT gene expression data, individuals with a high visceral fat gain appear to have reduced induction of expression of genes involved in triglyceride synthesis and lipid storage suggesting a reduced SAT lipid storage capacity in these individuals.

Testing this hypothesis further Fabbrini *et al.* overfed obese individuals who were either metabolically healthy *vs.* unhealthy[30]. It was hypothesised that the metabolically healthy obese (MHO) will be resistant, whereas the metabolically abnormal (MAO), will be prone to the adverse metabolic effects of overfeeding. Employing stable isotopes, the results demonstrated that metabolically healthy obese, but not metabolically unhealthy obese, were protected from the adverse metabolic effects from weight gain with no change in hepatic and peripheral insulin sensitivity or in VLDL-TG secretion rates with overfeeding. This was related to upregulation of biological pathways and genes assoicated with AT lipogenesis in MHO, but not in MAO subjects. In contrast, McLaughlin *et al*, tested the hypothesis in obese, insulin-sensitve (IS) *vs.* obese insulin-resistant (IR) individuals postulating similarly that the IS subjects would demonstrate an adapative adipose cell/tissue and metabolic response. To the contrary, they found that IS, but not IR, subjects had greater

increases in VAT and liver fat and had a greater metabolic decompensation with overfeeding[31]. This metabolic decompensation was correlated with smaller baseline adipocyte size, greater adipocyte enlargement and decreased expression of lipid metabolism genes. Previously it was thought that adipocyte enlargement occurred due to increased triglyceride storage but the simultaneously reduced expression of lipid metabolism genes as cells enlarge suggests this was not the case. Rather, as with the study by Johannsen *et al.*[32], the influence of the baseline adipocyte cell size on worsening metabolic profiles suggest that adipocyte hypertrophy reflects impaired adipocyte differentiation faced with increased fat storage requirements. The explanation for these discrepant (and possibly counterintuitive) results are not clear, as the baseline characteristics of the two groups of study participants were not hugely dissimilar.

Votruba *et al.*, also investigated whether baseline insulin sensitivity could predict the pattern of weight change, hypothesising that insulin resistant individuals would accrue more abdominal subcutaneous or visceral fat whereas insulin sensitive individuals would accrue leg fat. No relationship was found between baseline insulin sensitivity and the pattern of regional fat distribution in response to overfeeding[33].

Intrinsic factors influencing the response to overfeeding

A number of studies highlight a significant genetic pre-disposition to the the relative amount and distribution of fat mass with overfeeding:

Twin studies Several twin studies have provided strong evidence that genetic factors significantly contribute to the individual differences in the sensitivity to alterations in energy balance. In the Quebec feeding study 12 pairs of monozygotic twins were overfed by 1000 kcal, six days a week for 84 days with a mean weight gain of 8.1kg

(2.7kg lean body mass). Although the range of weight gain between the twin pairs was staggering (4.3-13.3kg) with no correlation between the total energy ingested and weight gained, there was a high degree of concordance within each twin pair between the amount of weight gained and the distribution of excess energy[34].

Family history of type 2 diabetes mellitus (T2DM) Healthy individuals with a family history of T2DM are predisposed to the adverse effects of overfeeding. The response to overfeeding was studied in 41 sedentary individuals with and without a family history of T2DM (FH+ and FH- respectively). FH+ individuals gained more weight and became more insulin resistant[35].

Ethnicity It is well established that South Asians are more susceptible to central obesity and cardiometabolic consequences[36]. This maybe explained by their phenotype of higher fat mass and lower lean mass, contributing to insulin resistance[37, 38]. Overfeeding experiments with a short-term, high fat diet in South Asians *vs.* Caucasians has shown a more detrimental effect on the metabolic profile[39, 40].

Effect of low birth weight **Individuals with a low birth weight, despite their increased risk of insulin resistance when exposed to a high fat diet, did not differ in their AT response compared with control subjects[41].**

Participant characteristics Inter-individual differences in baseline characteristics explain varying weight change with factors such as low basal metabolic rate, lower baseline lipid oxidation (higher respiratory quotient, RQ), lower levels of spontaneous physical activity predisposing individuals to greater weight gain[42]. Baseline body weight and amount of body fat also determine the magnitude of the weight change and even for the same increment in energy intake these differ in lean and obese people.

Extrinsic factors influencing the response to overfeeding

Overfeeding regime characteristics The duration, energy density and the macronutrient composition of the overfeeding regime influences the response to overfeeding.

Effects of macronutrients A key consideration is the macronutrient composition of overfeeding and whether the effects differ depending on whether excess calories arise from high-fat, high-carbohydrate or a combination of both. This is particularly pertinent with conflicting public health messages about the relative merits and perils of high-fat or high-carbohydrate diets. Surprisingly, few studies have compared overfeeding regimens based on these macronutrients. Two studies characterised the effects of overfeeding with high fat *vs.* high carbohydrate diet on energy storage. Both showed comparable weight gain, however, Horton *et al* showed dietary fat to lead to greater fat accumulation than carbohydrate, whereas Lammert *et al* found there was no difference in fat storage based on macronutrient, explained by carbohydrates inducing hepatic and extrahepatic lipogenesis[2, 43]. Two small, short term studies have found fat and carbohydrate overfeeding to have similar effects on liver fat, however comprehensive assessment including molecular biology techniques and metabolic end-points is lacking [44, 45]. Bray *et al*. recently compared overfeeding regimes with different levels of dietary protein, finding the low protein group showed a greater increase in % body fat, but a decrease in intrahepatic lipid[46].

Influence of dietary fat composition In the LIPOGAIN study Rosqvist *et al*., overfed healthy individuals muffins with either polyunsaturated fatty acids (PUFA) or saturated fatty acids (SFA) and demonstrated distinct effects on the magnitude and distribution of fat deposition and on lean tissue[47]. With the PUFA diet equal amounts

of fat and lean tissue were added; in contrast, with a SFA diet four times as much fat as lean tissue was added.

Influence of dietary carbohydrate composition There has been interest in comparing the effects of different sugars on metabolic health, especially given a proposed link of excess fructose consumption with non-alcoholic fatty liver disease[48]. A small number of studies have compared fructose and glucose overfeeding. Two meta-analyses called for more data but found no difference in either lipid profile or ectopic fat deposits between different carbohydrate sources [49, 50].

Influence of pattern of feeding The effects of overfeeding differ according to the frequency and timing of the food intake. Overeating by consuming frequent meals (i.e. snacking) rather than isocaloric, large meals differentially affects the accumulation of intra-abdominal and liver fat [51].

Effects of overfeeding on other tissues/organs.

Skeletal muscle Effects in skeletal muscle have been examined and as in adipose tissue there is evidence of induction of extracellular matrix remodeling, inflammation, reduced insulin signaling and insulin resistance[27, 52] .

Cardiovascular system Increasing BMI is clearly linked with increasing risk of CVD[53] although individuals with metabolically healthy obesity may have some protection against it[54]. Similarly, normal weight individuals who are metabolically unhealthy (MUNW) also maybe at increased CV risk[15]. Cross-sectional mechanistic data involving detailed body composition and echocardiography shows that subclinical measures of systolic and diastolic myocardial performance are related to fat distribution and metabolic health rather than simply fat mass[21]. Metabolically healthy individuals, whether lean or obese, with lower VAT and liver fat have

preserved myocardial function compared with lean or obese, metabolically unhealthy individuals[21].

Effects of overfeeding on appetite and gut hormone regulation

Consistent with the concept of a weight 'set point', it has been speculated that a period of overfeeding may be accompanied by subsequent compensatory changes in peripheral signals from the gut or expanded adipose tissue mass that would help normalise body weight. Despite this there are few studies that have characterised alterations in the circulating levels of gut hormones or adipokines in response to overfeeding, nor to the modulation of appetite. The design, participants and results of these studies are summarized in Table 3.

Cornier *et al*., examined activation of key brain regions in response to visual food cues (control images, neutral hedonic value and high hedonic value food items) using functional MRI (fMRI). They studied participants after two days of eucaloric energy intake, followed by two days of overfeeding with 30% excess energy intake consumed. There was significant attenuation of the effect of the high hedonic value images after two days of overfeeding. Satiety ratings were also higher and hunger ratings lower after the overfeeding[55]. When comparing thin and reduced-obese individuals, the attenuation of the activation of brain regions by high hedonic value images after overfeeding was not observed in the reduced-obese individuals suggesting a propensity to gain weight[56]. Gut hormone responses have also been examined with conflicting results (Table 3).

Interaction of overfeeding with changes in physical activity

Few studies have examined the interaction of changes in physical activity with overfeeding. Knudsen *et al.*, implemented a 14 day overfeeding protocol (total energy intake increased by ~50%) combined with physical inactivity (step reduction to 1,500 steps/day) in healthy young men[57]. Changes in insulin sensitivity were apparent prior to changes in body composition measured by DEXA/MRI[57]. Wahlin implemented a similar protocol for 7 days, with an overconsumption of 50% excess energy simultaneously restricting the physical activity to below 4,000 steps, and similarly noted a dramatic reduction in insulin sensitivity with modulation of key metabolic genes (e.g. SREBP1c and FAS) and protein expression (GLUT4, AMPK, AKT1 and AKT2) within adipose tissue[58]. Significantly, the same short-term overfeeding and reduced physical activity protocol, with inclusion of 45 min of daily treadmill running at 70% maximal oxygen uptake, counteracted most of the detrimental effects at a whole-body and adipose tissue level, despite the provision of additional dietary energy intake to account for the extra energy expended by exercise[58].

Conclusions and future lines of research

The challenge with the current obesity epidemic is to understand how to facilitate healthy AT remodeling expansion with hyperplasia, involving adipocyte differentiation, rather than dysfunctional AT remodeling with hypertrophy, induction of insulin resistance and inflammation. In doing so we can reduce ectopic fat and potentially ectopic fat-related complications, T2DM, NAFLD and CVD. Prediction of personal fat thresholds would help individuals maintain their metabolic health as long as possible. Overfeeding studies using drugs that cause SAT proliferation (e.g. thiazolidinediones) to facilitate healthy AT expansion and partition excess lipid in the SAT may provide useful insight. This review has highlighted the paucity of knowledge regarding adipose tissue, metabolic and cardiovascular responses to excess

calories from fat *vs.* carbohydrate intake. This area is a major concern for public health and appropriate dietary recommendations and is a knowledge void that needs filling.

Conflict of Interest

The authors declare no conflict of interest.

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Figure legends

Table 1 Overview of feeding studies detailing baseline participant characteristics and overfeeding regime summarising those using concomitant assessment of body composition (*DEXA ± MRI ±CT*) to determine fate of excess energy into regional fat depots. F Fat; CHO Carbohydrate; NAFLD Non-Alcoholic Fatty Liver Disease.

Table 2 Key studies examining adipose tissue deposition, changes in adipose tissue structure/biology and metabolic consequences following overfeeding. IHTG Intrahepatic triglycerides; TG Triglycerides; HOMA-IR Homeostatic Model Assessment- Insulin Resistance; NEFA Non-esterified Fatty Acids; SAT Subcutaneous Adipose Tissue; AUC Area Under Curve; FFA Free Fatty Acids; VLDL Very Low Density Lipoproteins; IMCL Intramyocellular Lipids; IS Insulin Sensitivity

Table 3 Key studies examining changes in appetite or circulating levels of adipokines/gut hormones in response to overfeeding. CHO Carbohydrate; F Fat; P Protein; VAS Visual Analogue Scales; fMRI functional Magnetic Resonance Imaging; PYY Peptide YY; GLP-1 Glucagon-like peptide-1.

Figure 1 Conceptual framework highlighting potential mechanisms where inter-individual differences in partitioning of excess energy with overfeeding may arise. Inter-individual differences may arise due to **A)** proportion of excess energy expended *vs.* excess energy stored, **B)** relative storage in adipose tissue *vs.* in lean body mass, **C)** relative storage in upper body *vs.* lower body fat, **D)** amount of ectopic fat

deposition in visceral adipose tissue (VAT), liver or other organs (skeletal muscle, heart or pancreas etc.).

Figure 2 The relationship between BMI and insulin sensitivity is not linear as suggested by epidemiological evidence. Rather individuals are susceptible to metabolic decompensation when their weight exceeds their '*personal fat threshold*'. This threshold varies hugely: those with a low 'personal fat threshold' are more susceptible to cardio-metabolic decompensation with only modest weight gain (metabolically unhealthy normal weight) *vs*. a higher threshold means individuals can withstand much greater weight gain without decompensating (metabolically healthy obese) (adapted from Taylor *et al*.[19]).

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Table 1

Reference	Baseline characteristics	Mean Age (y)	Mean BMI (kg/m^2)	Overfeeding regime	Period	Activity	Body composition analysis modality
Van der Meer *et al.* 2008[59]	15 healthy men	25±6.6	23.4±2.5	Normal diet + 2632 kcal/d; 94% F	3 days	Free living	Cardiac and liver ^{1}H-MRS
Tchoukalova *et al.* 2010[13] and Votruba *et al.* [30]	28 healthy men (n=15), women (n=13)	NR	22.1±0.5	Tailored to achieve 5% weight gain	56 days	Free living	DEXA CT at L2/3, L3/4 and L4/5.
Sevastianova *et al.* 2012[60]	17 non-diabetic males (n=5), females (n=11) (56% with NAFLD)	Median 54 (40-59)	30.6±1.2	Normal diet + 1000kcal/d; 98% CHO	21 days	Free living	Abdominal MRI (T1-weighted) Liver ^{1}H-MRS
Alligier *et al.* 2012,2013[25, 29]	44 healthy men	33±1	NR (range 18-30)	Regular diet + 760kcal/d; 91% F	56 days	Usual	DEXA Abdominal MRI (T1-weighted)
Knudsen *et al.* 2012[57]	9 healthy men	24±3.3	21.6-±2.5	Usual diet + 1500kcal as snack packages	14 days	Step reduction <1500 steps/day (10278±2399 to 1521±488)	DEXA/Abdominal MRI
Koopman *et al.* 2014[51]	36 healthy men, 4 groups: HFHS-S n=8 HFHS-F n=8 HS-S n=10 HS-F n=10	 22.6±2.9 21.5±1.9 22±2.5 21.9±2.8	 22.3±1 22.5±1.5 21.7±1.1 22.6±1.8	140% BL requirement: increased meal size (S) or frequency (F). Two supplements: High Fat High Sugar (HFHS): 49% CHO, 35% F, 16% P High Sugar (HS): Commercial sucrose drinks.	42 days	Free living	Abdominal MRI (T1-weighted) Liver ^{1}H-MRS
Johannsen *et al.* 2014[32]	29 healthy men	26.8±5.4	25.5±2.3	1.4X BL energy requirement; 41% CHO, 44% F, 15% P.	56 days	Free living	Abdominal MRI (T1-weighted) ^{1}H-MRS of liver and soleus muscle
Rosqvist *et al* . 2014[47]	39 healthy subjects: PUFA intervention: 5 women, 13 men SFA intervention: 6 women, 13 men	 PUFA: 26.7±4.6 SFA: 27.1±3.6	 PUFA: 20.8 (19.5-23.1) SFA: 19.9 (18.9-20.7)	Regular diet + muffins (51% F, 5% P, 44% CHO) titrate to weight gain supplemented with polyunsaturated (PUFA) or saturated (SFA) fat	49 days	Usual	Abdominal MRI ^{1}H-MRS liver Pancreatic MRS
Fabbrini *et al.* 2015[30]	20 obese subjects: Metabolically normal (MNO; IHTG <5.6%) n=12 Metabolically abnormal (MAO; IHTG >10%) n=8	 MNO: 43±10 MAO: 52±7	 MNO: 34.0±3.0 MAO: 35.7±3.9	Regular diet +1000kcal/d maintaining macronutrient intake. Delivered via specific menu choices from fast food chains.	Until 5-7% weight gain; mean 52 days	Free living	Abdominal MRI (T1-weighted) Liver ^{1}H-MRS
Boon *et al* . 2015[61]	24 healthy men	22.1±0.4	21.5±0.4	Regular diet +1275kcal/d; 94% F	5 days	No physical activity	Liver ^{1}H-MRS
McLaughlin *et al* 2016[31]	15 insulin-sensitive 16 insulin resistant	54 ±8 57±6	29.3±2.4 30.7±2.7	Regular diet+ snacks/beverages Mean additional calories 880 kcal/d (50% CHO, 35% fat, 15% protein) Target weight gain 3.2 kg (0.8kg/week)	28 days	Free living	CT measured SAT, VAT and mid-thigh fat Liver ^{1}H-MRS

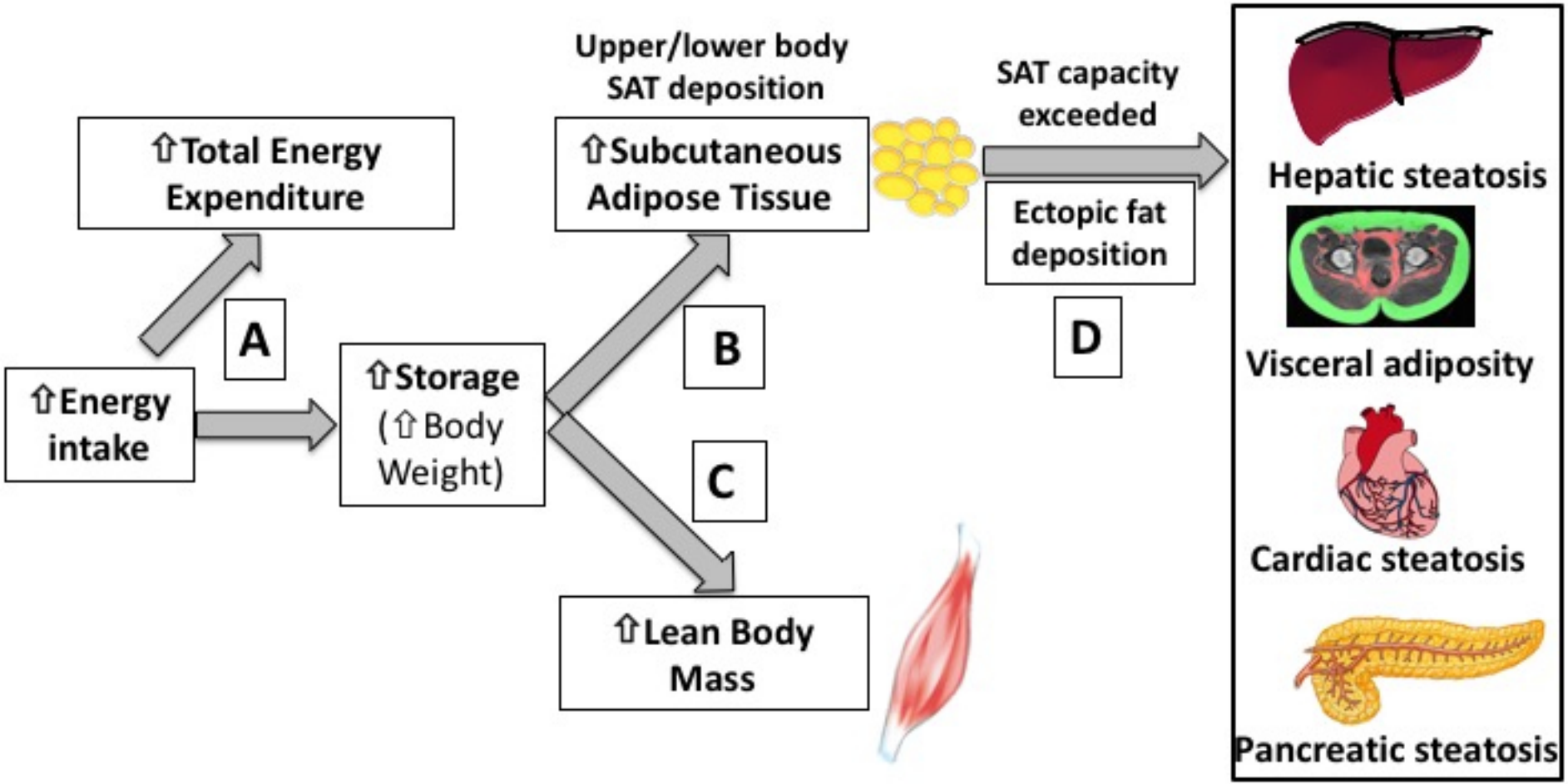
Table 2

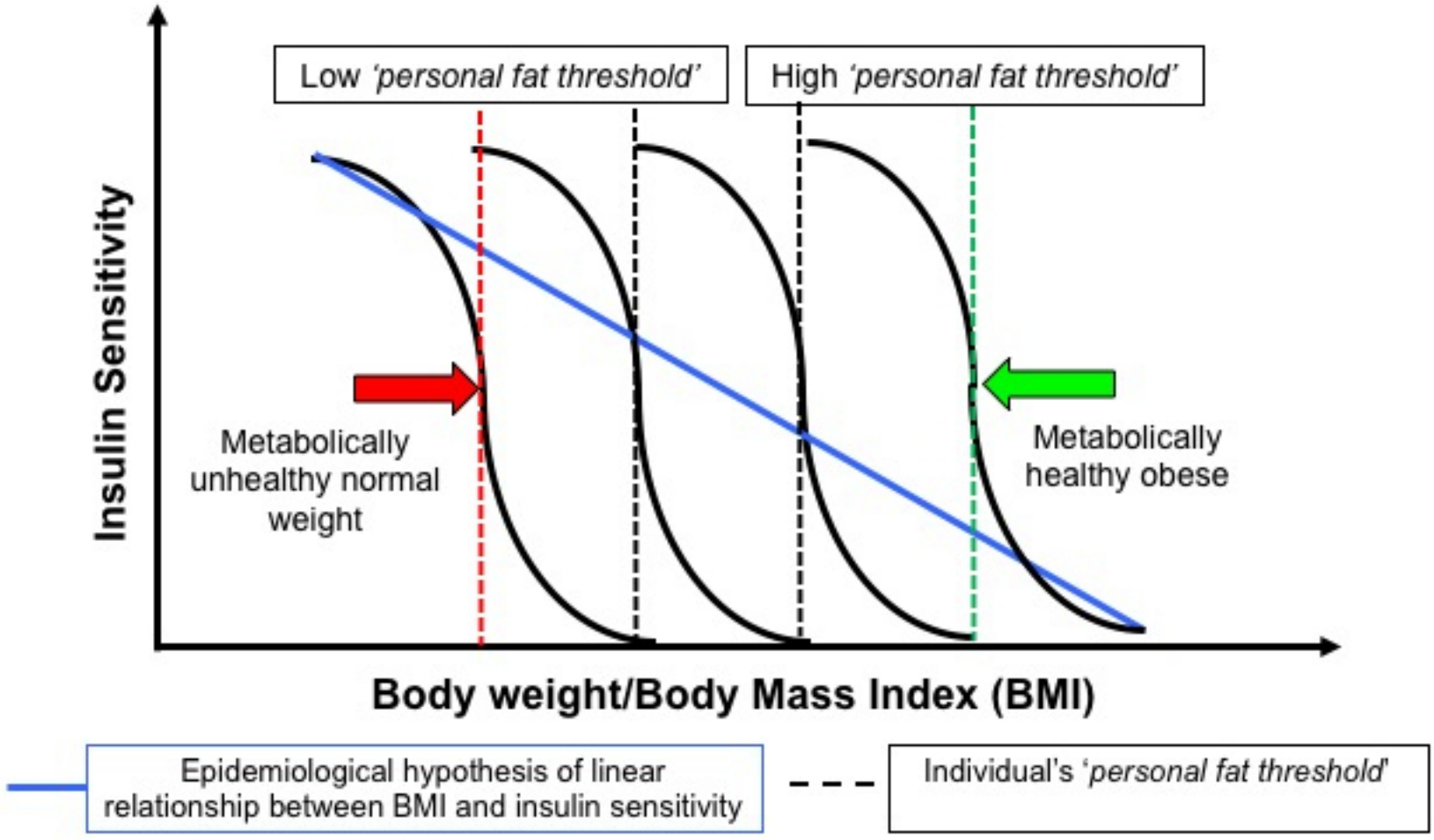
Reference	Weight gain (kg)	Changes in fat distribution			Adipocyte response	Metabolic response		Key findings
		Changes in SAT	Changes in VAT	Changes in liver fat		Insulin Sensitivity	Lipid levels	
Van der Meer *et al.* 2008[59]	BMI increased 23.4±2.5 to 23.6±2.5	NR	NR	**IHTG:** 2.01±1.79% to 4.26±2.78% **Cardiac TG:** 0.38±0.18% to 0.4±0.12%)	NA	**HOMA** 2.0±1.2 to 4.9±2.3	**TG** 1.3±0.4 to 2.9±1.1mmol/L **NEFA** 0.54±0.29 to 0.92±0.33mmol/L	NA
Tchoukolava *et al* 2010[13] **and Votruba *et al.***[30]	4.6±2.2kg	**Upper body:** +22.0±2.6% (women) +41.0±7.3% (men) **Lower body:** +18.2±1.3% (women) +34.9±5% (men)	+40.5%±5.8	NA	**Femoral/abdo SAT Size (µg lipid/cell):** Abdo: +39±11% Femoral: ±12±8% **No. ($x10^9$):** Upper body: +3±5% Lower body: +23±7%	**24 Insulin AUC** Increased by 2685±6252 (p=0.04).	NA	Abdominal SAT adipocyte size correlated with upper-body fat gain. No correlation between between baseline insulin sensitivity and upper body SAT or VAT gain.
Sevastianova *et al.*, 2012[60]	1.8±0.3kg (88.7±4.1 to 90.5±4.1kg)	4440 (3700-6210) to 4570 (4000-6280)cm^3	2180±300 to 2290±310cm^3	**IHTG:** 9.2±1.9% to 11.7±1.9%	NA	**HOMA-IR** 1.7±0.3 to 1.8-±0.2	**TG** 1.1±0.11 to 1.4±0.12; **FFA** 424±31 to 416±38 **Lipogenic index** 16:0/18:2n-6 ratio: **TG** 2.1 (1.9-2.3) to 2.6 (2.4-4.1) **VLDL** 2.1±-0.3 to 3.2±0.5	Increase in liver fat proportionate to de novo lipogenesis
Alligier *et al* 2012,2013[25, 29]	2.5kg 79.1±1.8 to 81.6±1.8kg	91±7 to 100±7cm^3	92±11 to 102±11cm^3	NA	**Abdominal SAT Size (cell surface µm2)** 3123±129 to 3120±160 **Number (cells/mm^2)** 320±16 to 336-±28	**HOMA-IR** 2.29±0.16 to 2.44±0.15	**FFA** (µM) 418±23 to 355±16	NA
Knudsen *et al* 2012[57]	1.6kg 71.3±3.5 to 72.9±3.4kg	NA	28.8±13.5 to 43.1±20.5cm^3	NA	NA	**HOMA-IR** 1.1 to 1.6 **OGTT AUC** increased 37±10% **Clamp: glucose infusion rate** reduced by 43.6±11%. **Matsuda index** reduced by 26±14%	**TG** 0.92 (0.64-1.3) to 1.13 (0.89-1.43) mM **FFA** 362.5(267.5-491.2) to 233.4 (138.5-393.1) µM	Reduction in insulin sensitivity precedes changes in body composition.
Koopman *et al* 2014[51]	POOLED HFHS/HS-S: BMI 22.05±0.98 to 22.75±1.04 POOLED HFHS/HS-F: BMI 22.5±1.5 to 23.2±1.6	POOLED HFHS/HS-S: 0.225±0.06 to 0.228-±0.056L POOLED HFHS/HS-F: 0.276±0.111 to 0.315±0.115L	0.196±0.068 to 0.215±0.041L 0.239±0.073 to 0.266±0.077L	Pooled HFHS/HS-S: **IHTG:** 0.83±0.38 to 1.00±0.78% Pooled HFHS/HS-F: **IHTG:** 1.22±0.93 to 2.18±1.9%	NA	**Clamp:** no change in peripheral insulin sensitivity.	**TG** significantly increased in HFHS-F group only (0.56±0.21 to 0.84±0.32mmol/L)	Hypercaloric diet with increased meal frequency increased intrahepatic fat independent of body weight gain and caloric content.
Johannsen *et al* 2014[32]	+7.6±2.1kg (81.9±10.3 to 89.5±-9.4kg)	**Abdominal SAT:** +1.3kg (4.1±1.5 to 5.4±1.8kg)	**Abdominal VAT:** +0.36kg (0.58±0.49 to 0.94±0.58kg)	**IHTG:** 1.5±0.6 to 2.19±1% **IMCL:** 0.45±0.24% to 0.49±0.24%	NA	**Clamp (**glucose infusion rate): **Low dose insulin:** +18% **High dose insulin:** +5% **EGP suppression:** 96±10% to 82±20%	**TG** (mg/dL) 87±42 to 96±68	Smaller adipocyte size associated with a greater decrease in insulin sensitivity. No association between adipocyte size and ectopic fat
Rosqvist *et al* 2014[47]	PUFA 1.6±0.85kg (BL 67.4kg) SFA 1.6±0.96kg (BL 63.3kg)	**Abdominal SAT:** PUFA +0.25±0.32L (baseline: 2.2L) SFA +0.34±0.23L (baseline: 1.8L)	PUFA +0.11±0.21L (baseline 0.99L) SFA +0.22±0.16L (baseline: 0.81L)	**IHTG:** PUFA +0.04±0.24% (baseline 0.75%) SFA +0.56±1% (baseline 0.96%)	NA	**HOMA-IR**: PUFA +0.2±-0.5 (baseline 1.23) SFA +0.18±0.3 (baseline 1.04)	NA	Changes in IHTG and VAT associated with changes in palmitic acid (SFA). Linoleic acid (PUFA) inversely associated with liver fat.

Fabbrini *et al* 2015[30]	MNO: +6%; 95.8±13.7 to 101.7±14.4kg MAO: +6%; 103±11 to 109±11.6kg	MNO: +2%; (3008±796 to 3071±809cm) MAO: +5%; 3145±871 to 3308±928cm^3	MNO: +12%; 885±240 to 987±295cm^3 MAO: +12%; 1714±585 to 1912±645cm3	**IHTG** MNO: 2.4±1.1 to 3.9±2.6% MAO: 15.2±4 to 22.8±4.3%	NA	**HOMA-IR**: MNO: +10% (baseline 2) MAO: +22% (baseline 6) **Clamp:** Suppression of glucose rate of appearance lower in MAO group.	**TG** (mg/dl): MNO: 0% (89±43 to 89±32) MAO +27% (134±61 to 170±52) **VLDL apoB100:** secretion increased in MAO but not MNO (p=0.004)	Transcriptional pathways related to lipid metabolism and synthesis: upregulated in metabolically healthy but not in metabolically unhealthy
Boon *et al* 2015[61]	69.1±1.9 to 69.6±1.9kg	NA	NA	**IHTG:** 1.57±0.27% to 3.43±0.49%	NA	**HOMA-IR:** 1.62±0.26 to 2.39±0.32	**TG** (mmol/l): 1.0±0.1 to 1.0±0.1 **NEFA** (mmol/l) 0.5±0.03 to 0.5±0.03	NA
McLaughlin *et al* 2016[31]	**IS** 86.2±10.1 to 89.6 ±10.3 **IR** 89.4±11.2 to 92.1±11.1	**IS:** 147 ± 54 to 162 ± 51cm^3 **IR:** 140 ± 34 to 148 ± 37cm^3	**IS:** 37±22 to 44±28cm^3 **IR:** 64±16 to 73±27cm^3	**IHTG: IS:** 0.03 ± 0.21 to 0.07 ± 0.04 **IHTG: IR:** 0.23±0.31 to 0.3±0.22	**Abdominal SAT size and structure:** Peak adipocyte diameter increased significantly only in IS subgroup. Significant decrease in percentage of small adipose cells in IS	**Muscle insulin resistance** worsened in IS group only: 45%(IS) *vs.* 8%(IR)	**Insulin suppression of lipolysis** worsened significantly in the IS subgroup alone	Smaller adipocyte size associated with a greater decrease in insulin sensitivity. IS rather than IR subjects experienced metabolic decompensation than IS subjects.

Table 3

Reference	Baseline characteristics	Mean Age (y)	Mean BMI (kg/m^2)	Dietary protocol	Period	Activity	Changes in appetite	Changes in gut hormones
Cornier et al, 2004[62]	13 thin (7 women, 6 men) and 9 reduced obese (RO; 5 women, 4 men) subjects. RO group underwent period of 10% weight loss then 4 weeks weight stability before study.	Thin: 30.6±8 (women) 29.3±7.6 (men). RO: 38.2±8.3 (women), 36.5±7.05 (men)	Thin: 20.6±1.8 (women) 21.3±3 (men). RO: 30.4±2.6 (women), 27.5±1.8 (men)	Eucaloric diet for 7 days followed by 50% overfeeding (50% CHO, 30% F, 20% P).	7 days eucaloric intake, 3 days overfeeding	Habitual physical activity	**VAS:** pre-meal hunger reduced in thin but not RO group following OF. Post meal satiety increased in thin but not RO group following OF. **Ad libitum energy intake:** following OF non-significantly reduced in all.	N/A
Jebb *et al*, 2006[63]	6 non-obese men	43.3 ± 10.6	21.9 ± 1.3	Overfeeding periods (+20%, +40%, +60% energy intake with fat) followed by free diet	3 x 3weeks	Habitual physical activity	**Food intake** stimulated overall during free diet period. Variable change with 'compensators' and 'non-compensators'.	**Leptin** elevated (+116%)
Cornier *et al* , 2007[55]	25 healthy men (n=12), women (n=13)	35.6 ± 6.2y vs. 33.8 ±4.7y	21.0 ± 1.3 vs. 22 ± 1.9	2 days eucaloric energy intake followed by 2 days overfeeding with 30% above eucaloric needs	2 days eucaloric intake, 2 days overfeeding	Habitual physical activity	**fMRI** response to visual food cues *(high hedonic value>neutral hedonic value)* blunted by overfeeding. **VAS:** reduced hunger and increased satiety ratings.	N/A
Cahill et al., 2011[64]	69 young men			70% more calories than required (15% protein, 35% fat and 50% carbohydrate	1 week	Not reported	N/A	**Serum PYY** concentration significantly increased in response to overfeeding
Wadden *et al* ., 2012[65]	68 young men (normal weight, n=26; overweight, n=14; obese, n=28)	23 ± 0.4y	25.6 ± 0.6	70% more calories than required (15% protein, 35% fat and 50% carbohydrate	1 week	Not reported	N/A	**Fasting serum acylated ghrelin** increased in all groups in response to overfeeding
Wadden *et al*., 2013[66]	72 healthy young men (normal weight n=30; overweight n=14; obese n=28)	23.11 ±0.37	25.27-±0.56	70% more calories than required (15% protein, 35% fat and 50% carbohydrate	1 week	Not reported	N/A	**Fasting GLP-1** increased in all groups with no difference based on weight status
Germain et al., 2014[67]**.**	8 constitutionally thin (CT) women (BMI <17.5 with no eating disorder or nutritional deficiency) and 8 normal weight controls	21.6±1.9 vs 22.1±0.8	17.1±0.3 vs 22.1±0.3	630kcal excess from fat (peanuts, cheese, olive oil, butter).	4 weeks	Habitual physical activity	N/A	**Incremental AUC for PYY and GLP-1** unchanged in CT group and decreased in normal weight group after overfeeding. Fasting ghrelin increased after overfeeding, lower in CT group vs normal weight.
Apolzan et al 2014[68]	15 men and 5 women. 1 normal weight, 8 overweight, 11 obese, otherwise healthy	34±9	30.7±4.6	140% energy requirements. 3 diets: High fat/low energy density (HF/LED; 1.05kcal/g; 50% F, 35% CHO, 15% P) , high fat/high energy density (HF/HED; 1.6kcal/g; 50% F, 35% CHO, 15% P), high carbohydrate/low energy density (HC/LED; 1.05kcal/g; 20% F, 65% CHO, 15% P)	3 arm cross over design: 2 days OF with 4 days measurement of ad libitum intake	Physical activity tailored so energy expenditure stable over study period.	**Ad libitum intake** higher on first day following OF compared with others. Trend towards lower than baseline ad libitum intake following OF (significant only in HF/LED group). VAS: decreased hunger and increased satiety following HF/LED overfeeding only.	N/A


⇧Total Energy Expenditure
A
⇧Energy intake
⇧Storage (⇧Body Weight)
B
C
Upper/lower body SAT deposition
⇧Subcutaneous Adipose Tissue
⇧Lean Body Mass
SAT capacity exceeded
Ectopic fat deposition
D
Hepatic steatosis
Visceral adiposity
Cardiac steatosis
Pancreatic steatosis


Low 'personal fat threshold'
High 'personal fat threshold'
Insulin Sensitivity
Metabolically unhealthy normal weight
Metabolically healthy obese
Body weight/Body Mass Index (BMI)
Epidemiological hypothesis of linear relationship between BMI and insulin sensitivity
Individual's 'personal fat threshold'