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 may be 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 gender/hormonal status, genetic/ethnic background, baseline metabolic health and cardiorespiratory fitness; extrinsic factors include macronutrient (fat *vs.* carbohydrate) content, fat/carbohydrate composition and overfeeding pattern.

Subcutaneous adipose tissue (SAT) analysis, coupled with metabolic assessment, with overfeeding have revealed how SAT remodels to accommodate excess nutrients. SAT remodelling occurs either by *hyperplasia* (increased adipocyte number) or by *hypertrophy* (increased adipocyte size). Biological responses of SAT also govern the extent of ectopic (visceral/liver) triglyceride 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 triglyceride 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 depot expansion/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 with disproportionally low physical activity coupled with increased energy intake (i.e. 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. When faced with energy excess, only 300-500g of carbohydrate can be stored as glycogen, thus any excess energy must either be oxidized or converted to triglyceride. In contrast to the other macronutrients, there is a virtually unlimited storage capacity for triglyceride within adipose tissue. Thus, body weight increase occurs predominantly via increased adipose tissue volume with a small increase in fat-free mass[3].

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 state of (at least) short-term energy surplus 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. For ethical reasons, these studies are usually short- to medium-term, ranging in duration from <24 hours to 8-12 weeks.

Review Methodology

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. This was supplemented by manual reference searches: 2272 abstracts were screened, with 168 articles reporting the effects of hypercaloric diets in humans identified.

This review is limited to describing studies in which hypercaloric diets were used in an experimental setting in human subjects. No exclusion is made based on the source of additional energy, however studies that overfed subjects with one macro or micronutrient but no overall caloric excess are not considered. No exclusions are made based on participant characteristics or co-morbidity and study design.

In order to assist with direct interpretation of data, the study design, participants and results from studies meeting the inclusion criteria and assessing current key areas of interest are described in tables. Specifically, these deal with the effect of overfeeding on adipose tissue and ectopic fat distribution, adipocyte and metabolic responses (Tables 1 & 2) and on adipokines, gut hormones and appetite regulation (Table 3).

Terminology used in the review

The term *fat* in this review refers to the dietary macronutrient. *Adipose tissue* is the anatomical term for the loose connective tissue, the main cell type being the *adipocyte*; adipose tissue also contains the stromal vascular fraction consisting of pre-adipocytes, fibroblasts, vascular endothelial cells and various immune cells including macrophages. Adipose tissue maybe stored as *Subcutaneous Adipose Tissue* (SAT) or as *Visceral Adipose Tissue* (VAT). *Adipocytes* are the cells that specialise in the synthesis and storage of *triglyceride*, esters composed of glycerol and three fatty acids. Triglyceride deposition within the liver, i.e. intra-hepatic triglyceride is referred to as *liver fat*. *Lipogenesis* refers to fatty acid and triglyceride synthesis from glucose or other substrates.

Lessons learnt from early overfeeding studies

Forty years ago, to understand the biological response of adipose tissue to weight gain in terms of *hyperplasia* (i.e. increased adipocyte number) vs *hypertrophy* (i.e. increased adipocyte size), Sims *et al* conducted a landmark overfeeding study in inmates at Vermont State Prison[4]. 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 of 16.2 kg (21% mean increase; ~10.4 kg as fat), was a considerable inter-individual weight change (range, 9-19 kg; 15-25 % increase). Subjects followed their normal routine and their caloric content and dietary intake was carefully recorded. The findings highlighted that the magnitude of weight gain could not 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 adipocyte *hypertrophy* (increased cell size) rather than *hyperplasia* (increased cell number).

Genetic basis for variations in regional adipose tissue distribution and metabolic health

Adipose tissue 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 differences in distribution[5].

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 adipose tissue distribution[6, 7]. 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[6]. Conversely, genetic evidence has been provided for individuals with higher BMI but lower risk of T2DM, hypertension and CVD. Presence of such ‘favourable adiposity alleles’ are associated with lower insulin levels

and a higher SAT:VAT ratio (i.e. higher SAT capacity) [7]. The same genetic/epigenetic factors will also determine the pattern/distribution of adipose tissue 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 is predominantly stored in adipose tissue **(Figure 1B)** with a lesser amount as fat-free mass **(Figure 1C)**. SAT has been described as a 'metabolic sink', and once this sink is full, overflow of lipid from SAT to other sites occurs. The biological properties of subcutaneous adipose tissue, and its response to overfeeding, govern the distribution of adipose tissue change: upper *vs.* lower body fat *and* subcutaneous adipose tissue (SAT) *vs*. ectopic triglyceride deposition (as visceral adipose tissue (VAT), liver and pancreatic fat, intra and intermyocellular fat and perivascular 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 and ultimately on the development of atherosclerosis.

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[8]).

TEE TEE is stimulated with overfeeding (by ~10%)[9] but does not increase linearly with weight gain[10]. 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 adipose tissue volume *vs*. muscle mass (skeletal muscle has higher relative energy requirements relative to adipose tissue)[11].

The stimulation of REE also depends upon the macronutrient content of the overfeeding regime (discussed later) 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 adipose tissue 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 adiposity 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[12].

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[8]. 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 adipose tissue 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[8].

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; such models only require knowledge of age, height, body weight, gender and physical activity[11].

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 adipose tissue 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.* adipose tissue 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 adipose tissue *vs*. lean tissue[13].

Storage in upper body (abdominal) vs. lower body (gluteofemoral) adipose tissue The regional distribution of SAT, quantified by DEXA, is critically important with subcutaneous adipose tissue depots in upper and lower body characterized by structural and functional differences and therefore associated with different metabolic risk. Abdominal (i.e. upper body) SAT (ASAT) is characterized by high uptake of diet-derived fat and a high lipid turnover. In contrast, gluteofemoral adipose tissue (GFAT) has a reduced lipid turnover but a high capacity to accommodate lipids undergoing redistribution [14, 15].

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)[16]. Conversely, accumulation of adipose tissue in the lower body (gluteofemoral obesity) shows opposite associations with cardiovascular disease and type 2 diabetes mellitus when adjusted for overall adiposity. Paradoxically lower body adipose tissue accumulation is associated with improved cardiovascular and metabolic profiles (protective role) suggested to sequester lipids that would be destined for ectopic fat deposition[17].

Lower and upper body adipose tissue depots show a different response to weight gain reflecting their different biological characteristics and capacity for lipid storage/turnover[14].

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)[18]. 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* suggests that the capacity for AT expansion is determined by functional adipocyte characteristics and their molecular and biochemical adaptive responses to positive energy balance[19]. 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*[20]. This means that once the SAT storage capacity is reached, ectopic triglyceride deposition ensues with associated lipotoxicity and

metabolic dysfunction (Figure 2).

These concepts of a finite AT expandability, which has large inter-individual variation, may explain the distinct body composition phenotypes of metabolic healthy and unhealthy, lean or obese[21]. 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)[22]. 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)[21].

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 deposition (e.g. liver and visceral adipose tissue) and preserved insulin sensitivity with little/no systemic inflammation [23]. 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 consistent with observations in people with generalised lipodystrophy, who have limited capacity for subcutaneous adipose tissue storage and consequently develop severe insulin resistance, NAFLD and dyslipidaemia[24]. Conversely, the PPARϒ agonists, thiazolidinediones improve metabolic profiles by promoting a shift in fat distribution from visceral to subcutaneous fat depots, by stimulating adipogenesis within SAT[25].

Dysfunctional adipose tissue remodeling and metabolic consequences

AT remodeling involves recruitment of adipogenic precursor cells alongside induction of various other pathways including that of the renin-angiotensin pathway, angiogenesis and remodelling of the extracellular matrix[26]. In contrast, SAT expansion with limited angiogenesis and hypoxia results in secondary changes involving induction of tissue fibrosis[27], adipocyte cell death and enhanced pro-inflammatory cytokine secretion[28]. During this process there is a phenotypic switch with an greater infiltration of pro-inflammatory (M1) macrophages relative to the anti-inflammatory (M2) phenotype[29].

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 structural and functional adaptations of the adipocytes coupled with examination of regional adipose tissue depot expansion and partitioning of triglyceride 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 (e.g. abdominal obesity/increased waist circumference, 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[30]. 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 adipose

tissue. 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. Individuals with a high visceral adipose tissue gain appear to have reduced induction of expression of SAT genes involved in triglyceride synthesis and lipid storage[30]. Although gene expression changes, without concomitant measurements of protein expression/activity are not conclusive, these observations would be compatible with a reduced SAT lipid storage capacity in these individuals. Johannsen *et al.* noted a greater metabolic decompensation correlated with smaller baseline SAT adipocyte size which may suggest that adipocyte hypertrophy reflects impaired adipocyte differentiation when faced with increased fat storage requirements[33].

Testing this hypothesis further Fabbrini *et al.* overfed obese individuals who were either metabolically healthy *vs.* unhealthy[31]. 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. 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 same hypothesis in obese, insulin-sensitve (IS) *vs.* obese insulin-resistant (IR) individuals postulating similarly that the IS subjects would

demonstrate a superior adapative adipose cell/tissue and metabolic response[32]. To the contrary, they found that IS subjects had greater increases in VAT and liver fat and decompensation with overfeeding.

The explanation for these discrepant (and possibly counterintuitive) results between the overfeeding studies in individuals with different baseline metabolic health are not clear, but may relate to differences in baseline age, BMI and metabolic health, duration and nature of dietary intervention and the degree of weight gain.

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 adipose tissue 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[34].

Intrinsic factors influencing the response to 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 of weight gain within each twin pair. Furthermore there were significant within pair similarities in regional adipose tissue expansion and the ratio of abdominal to femoral adiposity, suggesting a strong genetic influence on both the amount and distribution of weight gain with overfeeding[35].

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[36].

Gender There are clear gender-specific differences in body composition with males more likely to accumulate central/abdominal fat and females to accumulate gluteofemoral adipose tissue[17]. Menopausal status also influences abdominal adipose tissue distribution with greater visceral adiposity in post *versus* pre-menopausal women[37]. The effects of gender and menopausal status will clearly influence the effects of overfeeding on regional adipose tissue deposition.

Ethnicity There are clear differences in adipose tissue distribution and phyisology according to ethnicity with Asians and Afro-Caribbeans having higher truncal fat mass, lower lean mass and dysfunctional adipose tissue compared with Europeans[38-41]. Thus, these ethnic groups are more susceptible to obesity-related cardiometabolic consequences, with incidence rates of type 2 diabetes equivalent to those with a BMI of 30 kg/m^2 occurring at much lesser obesity levels (whether using BMI or waist circumference) in South Asians, Chinese and African-Caribbeans [42, 43]. Overfeeding experiments with a short-term, high-fat diet in South Asians *vs.* Caucasians have not surprisingly shown more profound metabolic decompensation[44, 45].

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[46].

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[47]. Baseline body weight and adiposity 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 (discussed earlier within energy expenditure section). This is particularly pertinent with conflicting public health messages about the relative merits and perils of high-fat or high-carbohydrate diets. 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 overfeeding with excess dietary fat consumption led to greater relative adipose tissue accumulation than with excess dietary carbohydrate consumption[2]. In contrast, Lammert *et al* found similar degrees of adipose tissue accumulation with excess dietary fat or carbohydrate consumption; excess carbohydrates were converted to triglycerides by inducing hepatic and extra-hepatic lipogenesis[2, 48]. Two small, short-term studies found fat and carbohydrate overfeeding had similar effects on liver fat, however comprehensive assessment involving molecular biology techniques and metabolic end-points is lacking[49, 50]. 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[51].

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 adipose tissue deposition and on lean tissue[52]. With the PUFA diet, equal amounts of adipose and lean tissue were gained; in contrast, with a SFA diet four times as much adipose tissue as lean tissue was gained.

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[53]. 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 [54, 55].

Influence of pattern of feeding The effects of overfeeding differ according to the pattern of the food intake: overeating by consuming frequent meals (i.e. snacking) increased the accumulation of intra-abdominal and liver fat whereas larger meals (with an isocaloric intake) did not[56].

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[28, 57] .

Cardiovascular system Increasing BMI is clearly linked with increasing risk of CVD[58] although individuals with metabolically healthy obesity may have some protection against it[59]. Similarly, normal weight individuals who are metabolically

unhealthy (MUNW) also maybe at increased CV risk[16]. Cross-sectional mechanistic data involving detailed body composition and echocardiography shows that subclinical measures of systolic and diastolic myocardial performance are related to adipose tissue distribution and metabolic health rather than simply overall adiposity[22]. 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[22].

Effects of overfeeding on gut hormone, adipokines and appetite 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. Several studies have characterised alterations in circulating gut hormones, adipokines and the control of appetite after overfeeding (summarised in Table 3).

Cornier *et al.*, examined activation of key brain regions involved in appetite regulation, in response to visual food cues (control images, neutral hedonic and high hedonic value food items e.g. chocolate), using functional MRI. The authors studied participants after two days of eucaloric energy intake, followed by two days of overfeeding with 30% excess energy intake consumed. After two days of overfeeding, visualisation of high hedonic value images elicited lesser activation of these key appetite-regulating brain regions while after test meals satiety ratings were higher and hunger ratings lower (using visual analogue scales)[60]. These findings suggest homeostatic interactions occurred between overfeeding and subsequent regulation of energy intake. However, comparing thin and reduced-obese individuals (i.e. obese

individuals who had lost 8-10% of body weight through a weight-loss program), after overfeeding the neuronal response to high hedonic value images was reduced in thin but not in reduced-obese individuals[61]. Similarly, after overfeeding reduction in hunger ratings and increases in satiety ratings were less in reduced obese *versus* thin individuals[62]. These findings suggest adaptations in the reduced-obese individuals that would encourage weight regain.

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[63]. Changes in insulin sensitivity were apparent prior to changes in body composition measured by DEXA/MRI[63]. 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[64]. 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[64].

Confounding variables within overfeeding study designs.

This review highlights the numerous overfeeding studies performed; however, significant heterogeneity in study design, experimental technique and outcome

measures makes direct comparisons between studies difficult. Furthermore, there are a number of common limitations. Practical and ethical considerations mean that studies are generally small scale and short-term. Eliminating bias (including observer bias) is difficult and adjusting for confounding factors including physical activity, participant compliance and ensuring consistent delivery of overfeeding very challenging. Ideally, studies should be in controlled environments; however, this raises further technical, ethical and financial challenges.

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. Despite the numerous overfeeding studies performed, conclusions are hampered by significant heterogeneity in study design and the limited number of studies involving a controlled environment. However, such studies are technically and ethically difficult, with optimal study duration and design unclear, and the issue of controlling for confounding factors challenging. Considering such limitations, the fundamental question of adipose tissue, metabolic and cardiovascular responses to excess calories from fat *vs.* carbohydrate intake remains a major public health concern and is a knowledge void that needs filling with carefully designed interventions.

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*.[20]

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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[65]	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[14] **and Votruba *et al.*** [34]	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[66]	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[26, 30]	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[63]	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[56]	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[33]	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[52]	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[31]	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[67]	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[32]	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

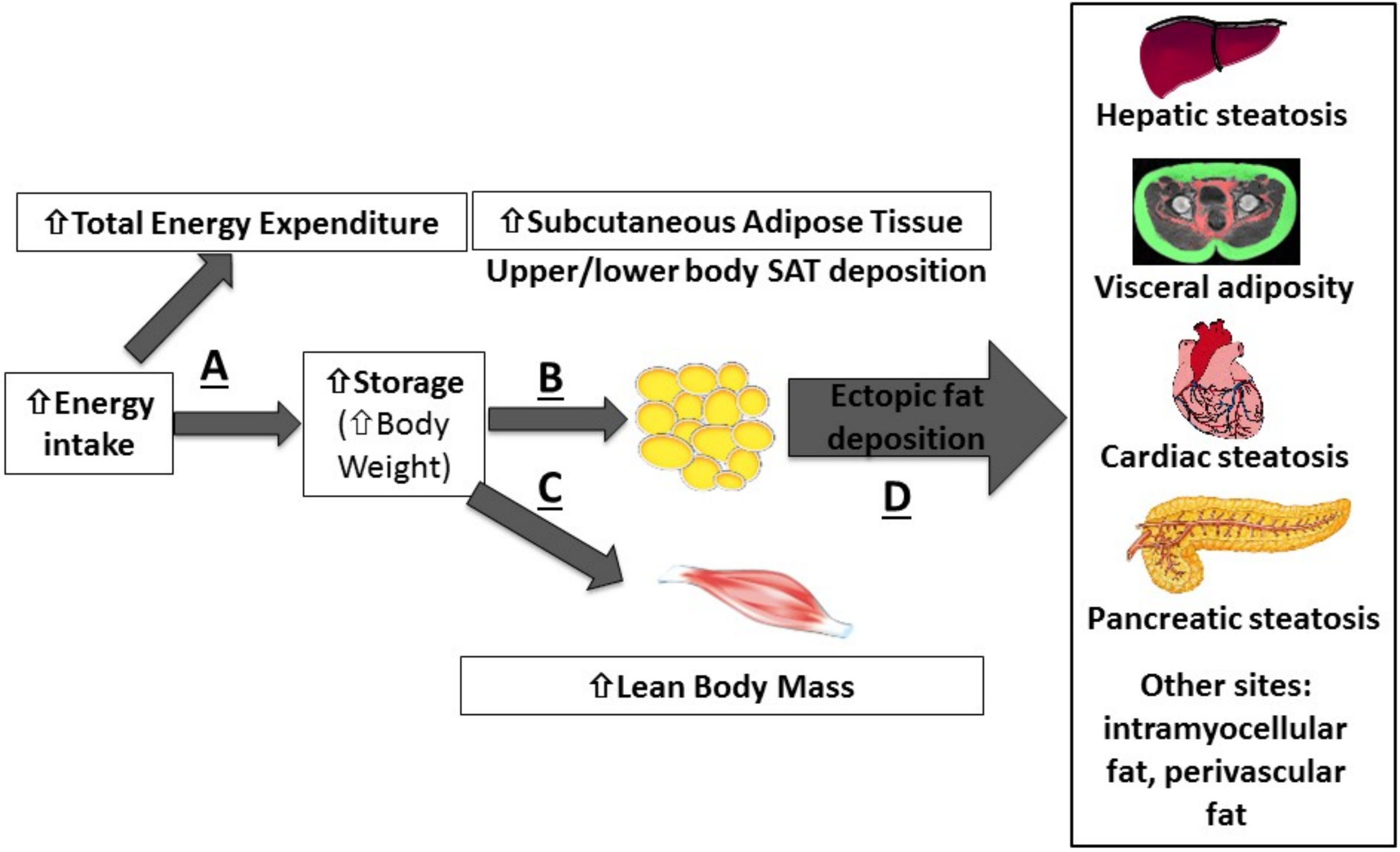
Table 2

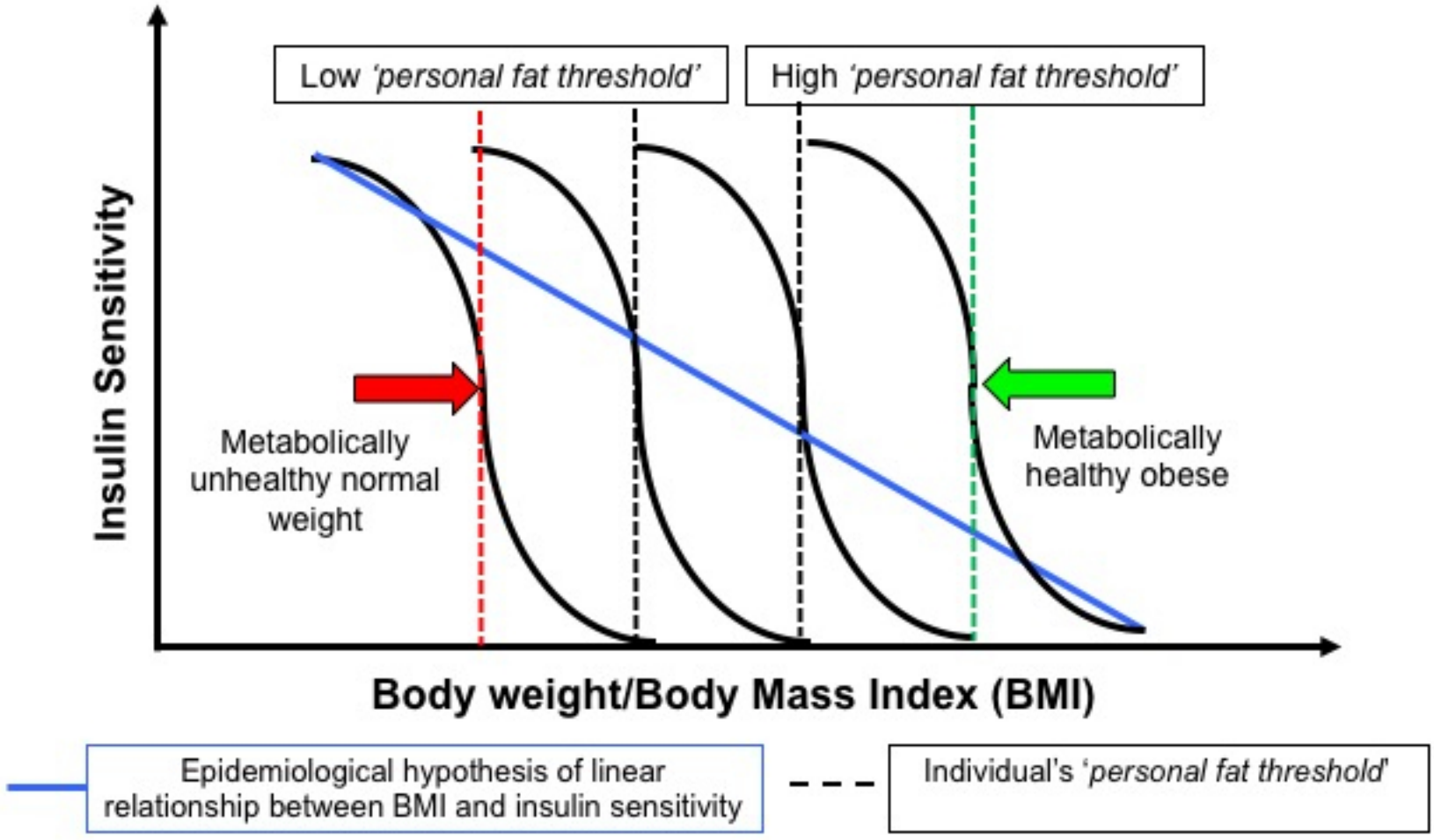
Reference	Weight gain (kg)	Changes in fat distribution			Adipocyte response	Metabolic response		Key findings
		Changes in SAT	Changes in VAT	Changes in liver fat		Insulin Sensitivity	Lipid levels	
Van der Meer *et al.* 2008[65]	BMI increased 23.4±2.5 to 23.6±2.5 Change in weight not reported	NR	NR	**IHTG:** 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[14] **and Votruba *et al.*** [34]	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[66]	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[26, 30]	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[63]	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[56]	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 Change in weight not reported	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[33]	+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[52]	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[31]	MNO: +6%; 95.8±13.7 to 101.7±14.4kg	MNO: +2%; (3008±796 to 3071±809cm)	MNO: +12%; 885±240 to 987±295cm^3	**IHTG** MNO: 2.4±1.1 to 3.9±2.6%	NA	**HOMA-IR**: MNO: +10% (baseline 2)	**TG** (mg/dl): MNO: 0% (89±43 to 89±32)	Transcriptional pathways related to lipid metabolism and synthesis: upregulated

	MAO: +6%; 103±11 to 109±11.6kg	MAO: +5%; 3145±871 to 3308±928cm^3	MAO: +12%; 1714±585 to 1912±645cm3	MAO: 15.2±4 to 22.8±4.3%		MAO: +22% (baseline-6) **Clamp:** Suppression of glucose rate of appearance lower in MAO group.	MAO +27% (134±61 to 170±52) **VLDL apoB100:** secretion increased in MAO but not MNO (p=0.004)	in metabolically healthy but not in metabolically unhealthy
Boon *et al* 2015[67]	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[32]	**IS** 86.2±10.1 to 89.6 ±10.3kg **IR** 89.4±11.2 to 92.1±11.1kg	**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	Weight gain	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	Not reported	**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[68]	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 periods	3 x 3weeks	Habitual physical activity	Total not reported Fat mass +3.3±1.6kg	**Food intake** stimulated overall during free diet period. Variable change with 'compensators' and 'non-compensators'.	**Leptin** elevated (+116%)
Cornier *et al* , 2007[61]	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	Not reported	**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[69]	69 young men (normal weight, n=27; overweight, n=14; obese n=28)	Normal weight: 23.7±3.6y Overweight: 22.0±3.1 Obese: 23.2±2.6	Normal weight: 22.6±2.6 Overweight: 24.1±1.3 Obese: 29.1±24.9	70% more calories than required (15% protein, 35% fat and 50% carbohydrate	1 week	Not reported	Normal weight: 72.4±9.2 to 74.5±9.6kg Overweight: 77.8±4.2 to 79.4±4.3kg Obese: 93.0±15.6 to 95.7±16kg	N/A	**Serum PYY** concentration significantly increased in response to overfeeding
Wadden *et al* ., 2012[70]	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	82.2±1.8 to 84.4±1.5kg	N/A	**Fasting serum acylated ghrelin** increased in all groups in response to overfeeding
Wadden *et al.*, 2013[71]	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	80.9±1.8 to 83.1±1.9kg	N/A	**Fasting GLP-1** increased in all groups with no difference based on weight status
Germain et al., 2014[72]	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	CT women +0.22± 0.18kg Controls +0.72± 0.26kg	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[3]	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.	HF/LED: +3.2±0.5kg HF/HED: +6.1±0.8kg HC/LED: 6.5±0.5kg	**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
⇧Subcutaneous Adipose Tissue
Upper/lower body SAT deposition
⇧Energy intake
A
⇧Storage (⇧Body Weight)
B
C
Ectopic fat deposition
D
⇧Lean Body Mass
Hepatic steatosis
Visceral adiposity
Cardiac steatosis
Pancreatic steatosis
Other sites: intramyocellular fat, perivascular fat


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'