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

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

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13

14                   **Running title:** Overfeeding, adipose expansion and metabolic risk

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25

1 **Abstract**

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

14 Intrinsic and extrinsic factors modulate individuals' overfeeding response: intrinsic  
15 factors include gender/hormonal status, genetic/ethnic background, baseline metabolic  
16 health and cardiorespiratory fitness; extrinsic factors include macronutrient (fat *vs.*  
17 carbohydrate) content, fat/carbohydrate composition and overfeeding pattern.

18 Subcutaneous adipose tissue (SAT) analysis, coupled with metabolic assessment, with  
19 overfeeding have revealed how SAT remodels to accommodate excess nutrients. SAT  
20 remodelling occurs either by *hyperplasia* (increased adipocyte number) or by  
21 *hypertrophy* (increased adipocyte size). Biological responses of SAT also govern the  
22 extent of ectopic (visceral/liver) triglyceride deposition. Body composition analysis  
23 by DEXA/MRI have determined the relative expansion of SAT (including  
24 abdominal/gluteofemoral SAT) *versus* ectopic fat with overfeeding.

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

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

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

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## 2 **Introduction**

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

13 Obesity represents a state of energy imbalance created by mismatched energy  
14 expenditure with disproportionately low physical activity coupled with increased  
15 energy intake (i.e. nutrient excess). However, individuals subjected to a similar  
16 relative positive energy balance show considerable heterogeneity in the extent to  
17 which their body weight or body composition is altered. When faced with energy  
18 excess, only 300-500g of carbohydrate can be stored as glycogen, thus any excess  
19 energy must either be oxidized or converted to triglyceride. In contrast to the other  
20 macronutrients, there is a virtually unlimited storage capacity for triglyceride within  
21 adipose tissue. Thus, body weight increase occurs predominantly via increased  
22 adipose tissue volume with a small increase in fat-free mass<sup>3</sup>.

23 There is abundant information on weight loss (achieved in many different ways) but  
24 much less information on controlled weight gain. Overfeeding experiments in which  
25 we mimic a state of (at least) short-term energy surplus have facilitated our

1 understanding of the adaptive cellular, physiological and behavioral responses of  
2 adipose tissue and other organs (e.g. liver, skeletal muscle and brain) to weight gain  
3 and helped explain the inter-individual heterogeneity to weight gain. These studies  
4 have also provided insight into susceptibility to metabolic decompensation with  
5 weight gain. For ethical reasons, these studies are usually short- to medium-term,  
6 ranging in duration from <24 hours to 8-12 weeks.

7

## 8 **Review Methodology**

9 This is a narrative review, however, to ensure all relevant literature is considered,  
10 systematic searches were carried out on Medline and Scopus using the terms  
11 “overfeeding”, “overeating”, “hypercaloric”, “controlled weight gain” and  
12 “experimental weight gain” limited to English language papers with human subjects.  
13 This was supplemented by manual reference searches: 2272 abstracts were screened,  
14 with 168 articles reporting the effects of hypercaloric diets in humans identified.

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

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

25

## 26 **Terminology used in the review**

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

11

## 12 **Lessons learnt from early overfeeding studies**

13 Forty years ago, to understand the biological response of adipose tissue to weight gain  
14 in terms of *hyperplasia* (i.e. increased adipocyte number) vs *hypertrophy* (i.e.  
15 increased adipocyte size), Sims *et al* conducted a landmark overfeeding study in  
16 inmates at Vermont State Prison<sup>4</sup>. He studied 5 lean individuals, with no family  
17 history of obesity, and in exchange for early parole subjected them to 10 weeks of  
18 supervised overfeeding while they remained sedentary. They were fed a diet of their  
19 own choice consisting of a three-fold higher caloric intake than would be needed to  
20 maintain body weight, aiming for 15-25% weight gain.

21 Underlying the significant mean weight gain of 16.2 kg (21% mean increase; ~10.4  
22 kg as fat), was a considerable inter-individual weight change (range, 9-19 kg; 15-25  
23 % increase). Subjects followed their normal routine and their caloric content and  
24 dietary intake was carefully recorded. The findings highlighted that the magnitude of  
25 weight gain could not be predicted from the magnitude of positive calorie balance,  
26 with some individuals protected from, or predisposed to, weight gain through a

1 variety of mechanisms. The key finding was that fat mass expansion occurred via  
2 adipocyte *hypertrophy* (increased cell size) rather than *hyperplasia* (increased cell  
3 number).

#### 4 **Genetic basis for variations in regional adipose tissue distribution and metabolic** 5 **health**

6 Adipose tissue distribution appears intrinsic to the individual and is likely to depend  
7 on heritable factors such as genetic variants, which are likely also subject to  
8 epigenetic regulation. A recent study identified 49 genetic loci associated with waist-  
9 to-hip ratio (adjusted for BMI), showing a stronger effect in women. These loci were  
10 enriched for genes expressed in adipose tissue with pathway analysis implicating  
11 adipogenesis, angiogenesis and insulin resistance as processes influencing differences  
12 in distribution<sup>5</sup>.

13 Several recent publications have highlighted several specific (common) genetic  
14 variants (particularly those associated with insulin resistance) where there is  
15 dissociation between the body mass index (BMI) and the risk of type 2 diabetes  
16 mellitus (T2DM) or cardiovascular disease (CVD) based on differing body  
17 composition/regional adipose tissue distribution<sup>6, 7</sup>. Genetic evidence has been  
18 provided for normal weight/lower BMI individuals with a metabolically obese  
19 phenotype, incorporating components of the metabolic syndrome and whose body  
20 composition is characterised by greater hepatic steatosis and increased visceral  
21 adipose tissue (VAT) relative to subcutaneous adipose tissue (SAT) (i.e. lower SAT  
22 capacity). These individuals were at an increased risk of T2DM, coronary artery  
23 disease or hypertension<sup>6</sup>. Conversely, genetic evidence has been provided for  
24 individuals with higher BMI but lower risk of T2DM, hypertension and CVD.  
25 Presence of such 'favourable adiposity alleles' are associated with lower insulin levels

1 and a higher SAT:VAT ratio (i.e. higher SAT capacity) <sup>7</sup>. The same  
2 genetic/epigenetic factors will also determine the pattern/distribution of adipose tissue  
3 depot expansion during weight gain.

4

#### 5 **Conceptual framework for fate of excess energy** (Figure 1)

6 With overfeeding, there are two fates for the surplus energy: either through  
7 stimulation of energy expenditure or deposition in a storage depot (**Figure 1A**).  
8 However, the majority of excess energy is stored, rather than expended; the amount  
9 stored representing the difference between total energy expended and total energy  
10 ingested. The surplus energy is predominantly stored in adipose tissue (**Figure 1B**)  
11 with a lesser amount as fat-free mass (**Figure 1C**). SAT has been described as a  
12 ‘metabolic sink’, and once this sink is full, overflow of lipid from SAT to other sites  
13 occurs. The biological properties of subcutaneous adipose tissue, and its response to  
14 overfeeding, govern the distribution of adipose tissue change: upper vs. lower body fat  
15 *and* subcutaneous adipose tissue (SAT) vs. ectopic triglyceride deposition (as visceral  
16 adipose tissue (VAT), liver and pancreatic fat, intra and intermyocellular fat and  
17 perivascular fat) (**Figure 1D**). The distribution of excess body fat (whether stored as  
18 SAT, upper or lower body or as ectopic fat) has potentially profound secondary  
19 consequences on metabolic and cardiovascular risk and ultimately on the development  
20 of atherosclerosis.

21

#### 22 **Changes in energy expenditure with overfeeding** (Figure 1A)

23 Total energy expenditure (TEE) is composed of resting energy expenditure (REE)  
24 (~60% of total), thermic effects of food and activity energy expenditure (exercise and  
25 non-exercise activity thermogenesis<sup>8</sup>).

1 **TEE** TEE is stimulated with overfeeding (by ~10%)<sup>9</sup> but does not increase linearly  
2 with weight gain<sup>10</sup>. The extent of TEE stimulation during overfeeding governs the  
3 amount of excess energy stored and thus associated weight gain: individuals with a  
4 lesser tendency to gain weight increase TEE to a greater extent. With ensuing weight  
5 gain, resting metabolic rate will further increase (related to increased body mass) with  
6 recalibration dependent upon the relative changes in adipose tissue volume vs. muscle  
7 mass (skeletal muscle has higher relative energy requirements relative to adipose  
8 tissue)<sup>11</sup>.

9 The stimulation of REE also depends upon the macronutrient content of the  
10 overfeeding regime (discussed later) with a hierarchy of macronutrient oxidation;  
11 macronutrients with limited storage capacity are oxidized first. Fat overfeeding has  
12 minimal effect on fat oxidation and total energy expenditure, such that 90-95% of  
13 excess energy is stored, resulting in greater adipose tissue accumulation. In response  
14 to carbohydrate overfeeding, there is stimulation of carbohydrate oxidation and an  
15 increase in TEE with a lower proportion (75-85%) of energy stored<sup>2</sup>. Prolonged  
16 overfeeding carbohydrate increases body adiposity by stimulation of *de novo*  
17 *lipogenesis* of hepatic and extra-hepatic (adipose tissue) origin. The predominant  
18 effect of protein overfeeding is accretion of lean body mass with the effect of  
19 increasing resting metabolic rate<sup>12</sup>.

20 **Diet-induced thermogenesis (DIT)** DIT, the energy expenditure associated with  
21 metabolising food, is also influenced by both the energy content and the  
22 macronutrient composition of the food ingested: isocaloric amounts of protein,  
23 carbohydrate and fat increase diet-induced energy expenditure by 20-30%, 5-10% and  
24 0-3% of TEE respectively.

1 **Activity energy expenditure (AEE)** AEE is composed of energy expenditure related  
2 to spontaneous physical activity and non-exercise activity thermogenesis (NEAT).  
3 Differences in levels of NEAT have a greater impact on TEE than differences in  
4 spontaneous physical activity. Obese individuals tend to undertake less NEAT than  
5 lean individuals, being sedentary by a mean of 2 hours more per day<sup>8</sup>. NEAT has been  
6 shown to have a role in resistance to weight gain: individual susceptibility to  
7 overfeeding is determined by a variable induction in NEAT. 16 volunteers were  
8 overfed 1,000 calories daily for 2 months, with a mean weight gain of 10lb, but with a  
9 range of 2-16lb. Change in NEAT (kcal/day) was inversely correlated with adipose  
10 tissue gain (kg). Those with a high NEAT response were more protected from obesity  
11 with overfeeding; those with a low NEAT response were more susceptible to obesity  
12 with overfeeding<sup>8</sup>.

13

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

15 Weight gain during overfeeding cannot be oversimplified by assuming 3,500 calories  
16 equates to a 1lb/0.45kg change in body weight, even if the energy surplus during  
17 overfeeding is accurately quantified. This erroneous assumption is based upon the  
18 premise that body weight changes reflect primarily loss or gain of adipose tissue  
19 (comprising 87% triglyceride), knowing the energy density of fat to be 9 kcal/g.  
20 Longer term changes in body fat are accompanied by changes in lean tissue whose  
21 metabolisable energy density is significantly less than body fat (4 kcal/g). Increased  
22 lean body mass would increase REE and higher body weight increases the energy  
23 requirement of physical activity. Mathematical models of energy expenditure and  
24 weight change have been developed that reflect the dynamic changes in body

1 composition as weight increases; such models only require knowledge of age, height,  
2 body weight, gender and physical activity<sup>11</sup>.

3 A number of overfeeding studies have been performed with concomitant assessment  
4 of body composition by DEXA, CT and/or MRI to provide insight into which storage  
5 depot the excess energy is partitioned. Table 1 details the baseline participant  
6 characteristics and overfeeding regime used in overfeeding studies summarising those  
7 using concomitant assessment of body composition (*DEXA ± MRI*) to determine fate  
8 of excess energy into regional adipose tissue depots, with results summarized in Table  
9 2.

10 ***Storage in adipose tissue vs. in lean body mass*** The concept of energy partitioning  
11 relates to the proportion of excess energy that is directed towards lean tissue *vs.*  
12 adipose tissue with the energy partition ratio being a non-linear function of body fat.  
13 People with a higher initial body fat have a greater fraction of their weight change  
14 attributable to increases in adipose tissue *vs.* lean tissue<sup>13</sup>.

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

23 Accumulation of adipose tissue in the upper body (abdominal obesity) is associated  
24 with increased risk of development of insulin resistance, type 2 diabetes mellitus and  
25 higher cardiovascular and total mortality, independent of BMI. Indeed, individuals

1 with a normal BMI and abdominal obesity (determined by waist-hip ratio) have a  
2 higher mortality compared with either individuals with a normal BMI without central  
3 obesity or with all overweight or obese individuals (based on BMI)<sup>16</sup>. Conversely,  
4 accumulation of adipose tissue in the lower body (gluteofemoral obesity) shows  
5 opposite associations with cardiovascular disease and type 2 diabetes mellitus when  
6 adjusted for overall adiposity. Paradoxically lower body adipose tissue accumulation  
7 is associated with improved cardiovascular and metabolic profiles (protective role)  
8 suggested to sequester lipids that would be destined for ectopic fat deposition<sup>17</sup>.

9 Lower and upper body adipose tissue depots show a different response to weight gain  
10 reflecting their different biological characteristics and capacity for lipid  
11 storage/turnover<sup>14</sup>.

12 ***Storage in subcutaneous adipose tissue vs. ectopic fat deposition (visceral adipose***  
13 ***tissue and liver)*** Subcutaneous adipose tissue (SAT) must undergo expansion to  
14 accommodate increased lipid supply to avoid deposition of lipids/fatty acids in non-  
15 adipocyte cells (causing lipotoxicity)<sup>18</sup>. SAT expansion may occur by two distinct  
16 mechanisms: *hypertrophy* of existing adipocytes or promotion of differentiation of  
17 pre-adipocytes (*hyperplasia*).

18 The *adipose tissue expandability hypothesis* suggests that the capacity for AT  
19 expansion is determined by functional adipocyte characteristics and their molecular  
20 and biochemical adaptive responses to positive energy balance<sup>19</sup>. This capacity is  
21 limited and determines the propensity for excess lipids to be orientated to other tissues  
22 *i.e.* ectopic lipid deposition, with secondary lipotoxicity. Taylor *et al.*, proposed a  
23 large inter-individual variation in the SAT buffering capacity with each individual  
24 having a *personal fat threshold*<sup>20</sup>. This means that once the SAT storage capacity is  
25 reached, ectopic triglyceride deposition ensues with associated lipotoxicity and

1 metabolic dysfunction (Figure 2).

2 These concepts of a finite AT expandability, which has large inter-individual  
3 variation, may explain the distinct body composition phenotypes of metabolic healthy  
4 and unhealthy, lean or obese<sup>21</sup>. Body composition analysis from these individuals  
5 have confirmed that metabolically unhealthy normal weight individuals are  
6 characterised by a low capacity for SAT expandability (*low personal fat threshold*)  
7 hence their higher lipid deposition in other organs (resulting in a higher VAT:SAT  
8 ratio and higher liver fat)<sup>22</sup>. Conversely, metabolically healthy obese individuals are  
9 characterised by a high capacity for SAT expandability (*high personal fat threshold*)  
10 (a lower VAT:SAT ratio and lower liver fat content)<sup>21</sup>.

11 Insights from transgenic mice (lacking leptin while overexpressing adiponectin)  
12 demonstrate that massive expansion of SAT is metabolically inert, providing a safe  
13 harbor for potentially toxic lipids, with reduced ectopic deposition (e.g. liver and  
14 visceral adipose tissue) and preserved insulin sensitivity with little/no systemic  
15 inflammation<sup>23</sup>. In contrast, a reduced capacity for SAT expansion is associated with  
16 subsequent inflammatory consequences, development of systemic insulin resistance  
17 (IR) and metabolic syndrome (MS), associated with subsequent development of  
18 endothelial dysfunction and atherosclerosis. These findings are consistent with  
19 observations in people with generalised lipodystrophy, who have limited capacity for  
20 subcutaneous adipose tissue storage and consequently develop severe insulin  
21 resistance, NAFLD and dyslipidaemia<sup>24</sup>. Conversely, the PPAR $\gamma$  agonists,  
22 thiazolidinediones improve metabolic profiles by promoting a shift in fat distribution  
23 from visceral to subcutaneous fat depots, by stimulating adipogenesis within SAT<sup>25</sup>.

24 **Dysfunctional adipose tissue remodeling and metabolic consequences**

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

9 A number of overfeeding studies have tested the validity of the adipose tissue  
10 expandability hypothesis by concomitantly examining changes in adipose tissue  
11 (morphology, gene and protein expression), body composition (using DEXA and/or  
12 MRI/<sup>1</sup>H-MRS) and the metabolic consequences (using oral glucose tolerance test or  
13 euglycaemic clamps) (summarised in Table 2). Thus we are able to simultaneously  
14 examine structural and functional adaptations of the adipocytes coupled with  
15 examination of regional adipose tissue depot expansion and partitioning of  
16 triglyceride into different tissues (SAT *vs.* ectopic deposition). Such studies have  
17 provided mechanistic insight into how dysfunctional SAT remodeling contributes to  
18 visceral and liver fat deposition (clinically as non-alcoholic fatty liver disease,  
19 NAFLD) and in doing so initiating metabolic dysfunction with development of  
20 components of metabolic syndrome (e.g. abdominal obesity/increased waist  
21 circumference, dyslipidaemia, hypertension, insulin resistance).

22 Alligier *et al.* overfed participants an additional daily lipid mixture composed of 70g  
23 (760 kcal) of saturated and monounsaturated fatty acids for 56 days<sup>30</sup>. Mean body  
24 weight change was 2.5 kg with substantial inter-individual heterogeneity in magnitude  
25 of weight gain and in the relative accretion of subcutaneous *vs.* visceral adipose

1 tissue. Although the increment in SAT was associated with the increase in body  
2 weight, there was no relationship between the increment in body weight and VAT nor  
3 was there any association between the expansion of SAT and VAT volumes. The  
4 magnitude of the increase in VAT volume was positively correlated with the  
5 magnitude of the post-prandial exogenous fatty acid release in the circulation during a  
6 labelled palmitate test meal. Individuals with a high visceral adipose tissue gain  
7 appear to have reduced induction of expression of SAT genes involved in triglyceride  
8 synthesis and lipid storage<sup>30</sup>. Although gene expression changes, without concomitant  
9 measurements of protein expression/activity are not conclusive, these observations  
10 would be compatible with a reduced SAT lipid storage capacity in these individuals.  
11 Johannsen *et al.* noted a greater metabolic decompensation correlated with smaller  
12 baseline SAT adipocyte size which may suggest that adipocyte hypertrophy reflects  
13 impaired adipocyte differentiation when faced with increased fat storage  
14 requirements<sup>33</sup>.

15 Testing this hypothesis further Fabbrini *et al.* overfed obese individuals who were  
16 either metabolically healthy *vs.* unhealthy<sup>31</sup>. It was hypothesised that the  
17 metabolically healthy obese (MHO) will be resistant, whereas the metabolically  
18 abnormal (MAO), will be prone to the adverse metabolic effects of overfeeding. The  
19 results demonstrated that metabolically healthy obese, but not metabolically unhealthy  
20 obese, were protected from the adverse metabolic effects from weight gain with no  
21 change in hepatic and peripheral insulin sensitivity or in VLDL-TG secretion rates  
22 with overfeeding. This was related to upregulation of biological pathways and genes  
23 associated with AT lipogenesis in MHO, but not in MAO subjects. In contrast,  
24 McLaughlin *et al.*, tested the same hypothesis in obese, insulin-sensitive (IS) *vs.* obese  
25 insulin-resistant (IR) individuals postulating similarly that the IS subjects would

1 demonstrate a superior adaptive adipose cell/tissue and metabolic response<sup>32</sup>. To the  
2 contrary, they found that IS subjects had greater increases in VAT and liver fat and  
3 decompensation with overfeeding.

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

8 Votruba *et al.*, also investigated whether baseline insulin sensitivity could predict the  
9 pattern of weight change, hypothesising that insulin resistant individuals would accrue  
10 more abdominal subcutaneous or visceral adipose tissue whereas insulin sensitive  
11 individuals would accrue leg fat. No relationship was found between baseline insulin  
12 sensitivity and the pattern of regional fat distribution in response to overfeeding<sup>34</sup>.

13

#### 14 **Intrinsic factors influencing the response to overfeeding**

15 *Twin studies* Several twin studies have provided strong evidence that genetic factors  
16 significantly contribute to the individual differences in the sensitivity to alterations in  
17 energy balance. In the Quebec feeding study 12 pairs of monozygotic twins were  
18 overfed by 1000 kcal, six days a week for 84 days with a mean weight gain of 8.1kg  
19 (2.7kg lean body mass). Although the range of weight gain between the twin pairs  
20 was staggering (4.3-13.3kg) with no correlation between the total energy ingested and  
21 weight gained, there was a high degree of concordance of weight gain within each  
22 twin pair. Furthermore there were significant within pair similarities in regional  
23 adipose tissue expansion and the ratio of abdominal to femoral adiposity, suggesting a  
24 strong genetic influence on both the amount and distribution of weight gain with  
25 overfeeding<sup>35</sup>.

1 *Family history of type 2 diabetes mellitus (T2DM)* Healthy individuals with a family  
2 history of T2DM are predisposed to the adverse effects of overfeeding. The response  
3 to overfeeding was studied in 41 sedentary individuals with and without a family  
4 history of T2DM (FH+ and FH- respectively). FH+ individuals gained more weight  
5 and became more insulin resistant<sup>36</sup>.

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

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

21 *Effect of low birth weight* Individuals with a low birth weight, despite their increased  
22 risk of insulin resistance when exposed to a high fat diet, did not differ in their AT  
23 response compared with control subjects<sup>46</sup>.

24 *Participant characteristics* Inter-individual differences in baseline characteristics  
25 explain varying weight change with factors such as low basal metabolic rate, lower

1 baseline lipid oxidation (higher respiratory quotient, RQ), lower levels of spontaneous  
2 physical activity predisposing individuals to greater weight gain<sup>47</sup>. Baseline body  
3 weight and adiposity also determine the magnitude of the weight change and even for  
4 the same increment in energy intake these differ in lean and obese people.

5

## 6 **Extrinsic factors influencing the response to overfeeding**

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

10 *Effects of macronutrients* A key consideration is the macronutrient composition of  
11 overfeeding and whether the effects differ depending on whether excess calories arise  
12 from high-fat, high-carbohydrate or a combination of both (discussed earlier within  
13 energy expenditure section). This is particularly pertinent with conflicting public  
14 health messages about the relative merits and perils of high-fat or high-carbohydrate  
15 diets. Two studies characterised the effects of overfeeding with high fat vs. high  
16 carbohydrate diet on energy storage. Both showed comparable weight gain, however,  
17 Horton *et al* showed overfeeding with excess dietary fat consumption led to greater  
18 relative adipose tissue accumulation than with excess dietary carbohydrate  
19 consumption<sup>2</sup>. In contrast, Lammert *et al* found similar degrees of adipose tissue  
20 accumulation with excess dietary fat or carbohydrate consumption; excess  
21 carbohydrates were converted to triglycerides by inducing hepatic and extra-hepatic  
22 lipogenesis<sup>2, 48</sup>. Two small, short-term studies found fat and carbohydrate overfeeding  
23 had similar effects on liver fat, however comprehensive assessment involving  
24 molecular biology techniques and metabolic end-points is lacking<sup>49, 50</sup>. Bray *et al*.  
25 recently compared overfeeding regimes with different levels of dietary protein,

1 finding the low protein group showed a greater increase in % body fat, but a decrease  
2 in intrahepatic lipid<sup>51</sup>.

3 *Influence of dietary fat composition* In the LIPOGAIN study Rosqvist *et al.*, overfed  
4 healthy individuals muffins with either polyunsaturated fatty acids (PUFA) or  
5 saturated fatty acids (SFA) and demonstrated distinct effects on the magnitude and  
6 distribution of adipose tissue deposition and on lean tissue<sup>52</sup>. With the PUFA diet,  
7 equal amounts of adipose and lean tissue were gained; in contrast, with a SFA diet  
8 four times as much adipose tissue as lean tissue was gained.

9 *Influence of dietary carbohydrate composition* There has been interest in comparing  
10 the effects of different sugars on metabolic health, especially given a proposed link of  
11 excess fructose consumption with non-alcoholic fatty liver disease<sup>53</sup>. A small number  
12 of studies have compared fructose and glucose overfeeding. Two meta-analyses called  
13 for more data but found no difference in either lipid profile or ectopic fat deposits  
14 between different carbohydrate sources<sup>54, 55</sup>.

15 *Influence of pattern of feeding* The effects of overfeeding differ according to the  
16 pattern of the food intake: overeating by consuming frequent meals (i.e. snacking)  
17 increased the accumulation of intra-abdominal and liver fat whereas larger meals  
18 (with an isocaloric intake) did not<sup>56</sup>.

19

## 20 **Effects of overfeeding on other tissues/organs.**

21 *Skeletal muscle* Effects in skeletal muscle have been examined and as in adipose  
22 tissue there is evidence of induction of extracellular matrix remodeling, inflammation,  
23 reduced insulin signaling and insulin resistance<sup>28, 57</sup>.

24 *Cardiovascular system* Increasing BMI is clearly linked with increasing risk of  
25 CVD<sup>58</sup> although individuals with metabolically healthy obesity may have some  
26 protection against it<sup>59</sup>. Similarly, normal weight individuals who are metabolically

1 unhealthy (MUNW) also maybe at increased CV risk<sup>16</sup>. Cross-sectional mechanistic  
2 data involving detailed body composition and echocardiography shows that  
3 subclinical measures of systolic and diastolic myocardial performance are related to  
4 adipose tissue distribution and metabolic health rather than simply overall adiposity<sup>22</sup>.  
5 Metabolically healthy individuals, whether lean or obese, with lower VAT and liver  
6 fat have preserved myocardial function compared with lean or obese, metabolically  
7 unhealthy individuals<sup>22</sup>.

8

### 9 **Effects of overfeeding on gut hormone, adipokines and appetite regulation**

10 Consistent with the concept of a weight ‘set point’, it has been speculated that a  
11 period of overfeeding may be accompanied by subsequent compensatory changes in  
12 peripheral signals from the gut or expanded adipose tissue mass that would help  
13 normalise body weight. Several studies have characterised alterations in circulating  
14 gut hormones, adipokines and the control of appetite after overfeeding (summarised in  
15 Table 3).

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

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

7

### 8 **Interaction of overfeeding with changes in physical activity**

9 Few studies have examined the interaction of changes in physical activity with  
10 overfeeding. Knudsen *et al.*, implemented a 14 day overfeeding protocol (total energy  
11 intake increased by ~50%) combined with physical inactivity (step reduction to 1,500  
12 steps/day) in healthy young men<sup>63</sup>. Changes in insulin sensitivity were apparent prior  
13 to changes in body composition measured by DEXA/MRI<sup>63</sup>. Wahlin implemented a  
14 similar protocol for 7 days, with an overconsumption of 50% excess energy  
15 simultaneously restricting the physical activity to below 4,000 steps, and similarly  
16 noted a dramatic reduction in insulin sensitivity with modulation of key metabolic  
17 genes (e.g. SREBP1c and FAS) and protein expression (GLUT4, AMPK, AKT1 and  
18 AKT2) within adipose tissue<sup>64</sup>. Significantly, the same short-term overfeeding and  
19 reduced physical activity protocol, with inclusion of 45 min of daily treadmill running  
20 at 70% maximal oxygen uptake, counteracted most of the detrimental effects at a  
21 whole-body and adipose tissue level, despite the provision of additional dietary  
22 energy intake to account for the extra energy expended by exercise<sup>64</sup>.

23

### 24 **Confounding variables within overfeeding study designs.**

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

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

8

### 9 **Conclusions and future lines of research**

10 The challenge with the current obesity epidemic is to understand how to facilitate  
11 healthy AT remodeling expansion with hyperplasia, involving adipocyte  
12 differentiation, rather than dysfunctional AT remodeling with hypertrophy, induction  
13 of insulin resistance and inflammation. In doing so we can reduce ectopic fat and  
14 potentially ectopic fat-related complications, T2DM, NAFLD and CVD. Prediction of  
15 personal fat thresholds would help individuals maintain their metabolic health as long  
16 as possible. Despite the numerous overfeeding studies performed, conclusions are  
17 hampered by significant heterogeneity in study design and the limited number of  
18 studies involving a controlled environment. However, such studies are technically and  
19 ethically difficult, with optimal study duration and design unclear, and the issue of  
20 controlling for confounding factors challenging. Considering such limitations, the  
21 fundamental question of adipose tissue, metabolic and cardiovascular responses to  
22 excess calories from fat vs. carbohydrate intake remains a major public health concern  
23 and is a knowledge void that needs filling with carefully designed interventions.

24

### 25 **Conflict of Interest**

26 The authors declare no conflict of interest.

## References

1. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A *et al.* Long-term persistence of hormonal adaptations to weight loss. *N Engl J Med* 2011; **365**(17): 1597-604.
2. Horton TJ, Drougas H, Brachey A, Reed GW, Peters JC, Hill JO. Fat and carbohydrate overfeeding in humans: different effects on energy storage. *Am J Clin Nutr* 1995; **62**(1): 19-29.
3. Apolzan JW, Bray GA, Smith SR, de Jonge L, Rood J, Han H *et al.* Effects of weight gain induced by controlled overfeeding on physical activity. *Am J Physiol Endocrinol Metab* 2014; **307**(11): E1030-7.
4. Salans LB, Horton ES, Sims EA. Experimental obesity in man: cellular character of the adipose tissue. *J Clin Invest* 1971; **50**(5): 1005-11.
5. Shungin D, Winkler TW, Croteau-Chonka DC, Ferreira T, Locke AE, Mägi R *et al.* New genetic loci link adipose and insulin biology to body fat distribution. *Nature* 2015; **518**(7538): 187-96.
6. Yaghoobkar H, Scott RA, White CC, Zhang W, Speliotes E, Munroe PB *et al.* Genetic evidence for a normal-weight "metabolically obese" phenotype linking insulin resistance, hypertension, coronary artery disease, and type 2 diabetes. *Diabetes* 2014; **63**(12): 4369-77.
7. Yaghoobkar H, Lotta LA, Tyrrell J, Smit RA, Jones SE, Donnelly L *et al.* Genetic evidence for a link between favorable adiposity and lower risk of type 2 diabetes, hypertension and heart disease. *Diabetes* 2016.
8. Levine JA, Lanningham-Foster LM, McCrady SK, Krizan AC, Olson LR, Kane PH *et al.* Interindividual variation in posture allocation: possible role in human obesity. *Science* 2005; **307**(5709): 584-6.
9. Diaz EO, Prentice AM, Goldberg GR, Murgatroyd PR, Coward WA. Metabolic response to experimental overfeeding in lean and overweight healthy volunteers. *The American journal of clinical nutrition* 1992; **56**(4): 641-55.
10. Harris AM, Jensen MD, Levine JA. Weekly changes in basal metabolic rate with eight weeks of overfeeding. *Obesity (Silver Spring)* 2006; **14**(4): 690-5.
11. Hall KD, Sacks G, Chandramohan D, Chow CC, Wang YC, Gortmaker SL *et al.* Quantification of the effect of energy imbalance on bodyweight. *Lancet* 2011; **378**(9793): 826-37.
12. Bray GA, Smith SR, de Jonge L, Xie H, Rood J, Martin CK *et al.* Effect of dietary protein content on weight gain, energy expenditure, and body composition during overeating: a randomized controlled trial. *Journal of American Medical Association* 2012; **307**(1): 47-55.

13. Hall K. Modeling Metabolic Adaptations and Energy Regulation in Humans. *Annual Review Nutrition* 2012; **32**: 35-54.
14. Tchoukalova YD, Votruba SB, Tchkonina T, Giorgadze N, Kirkland JL, Jensen MD. Regional differences in cellular mechanisms of adipose tissue gain with overfeeding. *Proceedings of the National Academy of Sciences of the United States of America* 2010; **107**(42): 18226-31.
15. Pinnick KE, Nicholson G, Manolopoulos KN, McQuaid SE, Valet P, Frayn KN *et al.* Distinct developmental profile of lower-body adipose tissue defines resistance against obesity-associated metabolic complications. *Diabetes* 2014; **63**(11): 3785-97.
16. Sahakyan KR, Somers VK, Rodriguez-Escudero JP, Hodge DO, Carter RE, Sochor O *et al.* Normal-Weight Central Obesity: Implications for Total and Cardiovascular Mortality. *Ann Intern Med* 2015; **163**(11): 827-35.
17. Karpe F, Pinnick KE. Biology of upper-body and lower-body adipose tissue-link to whole-body phenotypes. *Nat Rev Endocrinol* 2014; **11**(2): 90-100.
18. Rutkowski JM, Stern JH, Scherer PE. The cell biology of fat expansion. *Journal of Cell Biology* 2015; **208**(5)(1540-8140): 501-512.
19. Gray SL, Vidal-Puig AJ. Adipose tissue expandability in the maintenance of metabolic homeostasis. *Nutrition Reviews* 2007; **65**(6): S7-12.
20. Taylor R, Holman RR. Normal weight individuals who develop type 2 diabetes: the personal fat threshold. *Clin Sci (Lond)* 2015; **128**(7): 405-10.
21. Stefan N, Kantartzis K, Machann J, Schick F, Thamer C, Rittig K *et al.* Identification and characterization of metabolically benign obesity in humans. *Arch Intern Med* 2008; **168**(15): 1609-16.
22. Dobson R, Burgess MI, Sprung VS, Irwin A, Hamer M, Jones J *et al.* Metabolically healthy and unhealthy obesity: differential effects on myocardial function according to metabolic syndrome, rather than obesity. *Int J Obes (Lond)* 2016; **40**(1): 153-61.
23. Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM *et al.* Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *J Clin Invest* 2007; **117**(9): 2621-37.
24. Garg A. Acquired and inherited lipodystrophies. *N Engl J Med* 2004; **350**(12): 1220-34.
25. Spiegelman BM. PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. *Diabetes* 1998; **47**(4): 507-514.

26. Alligier M, Meugnier E, Debard C, Lambert-Porcheron S, Chanseau E, Sothier M *et al.* Subcutaneous adipose tissue remodeling during the initial phase of weight gain induced by overfeeding in humans. *The Journal of clinical endocrinology and metabolism* 2012; **97**(2): E183-92.
27. Kos K, Wong S, Tan B, Gummesson A, Jernas M, Franck N *et al.* Regulation of the fibrosis and angiogenesis promoter SPARC/osteonectin in human adipose tissue by weight change, leptin, insulin, and glucose. *Diabetes* 2009; **58**(8): 1780-8.
28. Tam CS, Covington JD, Bajpeyi S, Tchoukalova Y, Burk D, Johannsen DL *et al.* Weight gain reveals dramatic increases in skeletal muscle extracellular matrix remodeling. *J Clin Endocrinol Metab* 2014; **99**(5): 1749-57.
29. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *The Journal of clinical investigation* 2007; **117**(1): 175-84.
30. Alligier M, Gabert L, Meugnier E, Lambert-Porcheron S, Chanseau E, Pilleul F *et al.* Visceral fat accumulation during lipid overfeeding is related to subcutaneous adipose tissue characteristics in healthy men. *J Clin Endocrinol Metab* 2013; **98**(2): 802-10.
31. Fabbrini E, Yoshino J, Yoshino M, Magkos F, Tiemann Luecking C, Samovski D *et al.* Metabolically normal obese people are protected from adverse effects following weight gain. *J Clin Invest* 2015; **125**(2): 787-95.
32. McLaughlin T, Craig C, Liu L-F, Perelman D, Allister C, Spielman D *et al.* Adipose Cell Size and Regional Fat Deposition as Predictors of Metabolic Response to Overfeeding in Insulin-Resistant and Insulin-Sensitive Humans. *Diabetes* 2016; **65**(5): 1245-54.
33. Johannsen DL, Tchoukalova Y, Tam CS, Covington JD, Xie W, Schwarz JM *et al.* Effect of Eight Weeks of Overfeeding on Ectopic Fat Deposition and Insulin Sensitivity: Testing the "Adipose Tissue Expandability" Hypothesis. *Diabetes Care* 2014; **37**(10): 2789-97.
34. Votruba SB, Jensen MD. Insulin sensitivity and regional fat gain in response to overfeeding. *Obesity (Silver Spring)* 2011; **19**(2): 269-75.
35. Bouchard C, Tremblay A, Després J-P, Nadeau A, Lupien PJ, Thériault G *et al.* The Response to Long-Term Overfeeding in Identical Twins. *New England Journal of Medicine* 1990; **322**(21): 1477-1482.
36. Samocha-Bonet D, Campbell LV, Viardot A, Freund J, Tam CS, Greenfield JR *et al.* A family history of type 2 diabetes increases risk factors associated with overfeeding. *Diabetologia* 2010; **53**(8): 1700-8.
37. Kanaley JA, Sames C, Swisher L, Swick AG, Ploutz-Snyder LL, Stepan CM *et al.* Abdominal fat distribution in pre- and postmenopausal women: The

- impact of physical activity, age, and menopausal status. *Metabolism* 2001; **50(8)**: 976-982.
38. Sniderman AD, Bhopal R, Prabhakaran D, Sarrafzadegan N, Tchernof A. Why might South Asians be so susceptible to central obesity and its atherogenic consequences? The adipose tissue overflow hypothesis. *Int J Epidemiol* 2007; **36(1)**: 220-5.
  39. Chandalia M, Lin P, Seenivasan T, Livingston EH, Snell PG, Grundy SM *et al.* Insulin resistance and body fat distribution in South Asian men compared to Caucasian men. *PLoS One* 2007; **2(8)**: e812.
  40. Lear SA, Humphries KH, Kohli S, Chockalingam A, Frohlich JJ, Birmingham CL. Visceral adipose tissue accumulation differs according to ethnic background: results of the Multicultural Community Health Assessment Trial (M-CHAT). *Am J Clin Nutr* 2007; **86(2)**: 353-9.
  41. Lear SA, Kohli S, Bondy GP, Tchernof A, Sniderman AD. Ethnic variation in fat and lean body mass and the association with insulin resistance. *J Clin Endocrinol Metab* 2009; **94(12)**: 4696-702.
  42. Ntuk UE, Gill JM, Mackay DF, Sattar N, Pell JP. Ethnic-specific obesity cutoffs for diabetes risk: cross-sectional study of 490,288 UK biobank participants. *Diabetes Care* 2014; **37(9)**: 2500-7.
  43. Tillin T, Sattar N, Godsland IF, Hughes AD, Chaturvedi N, Forouhi NG. Ethnicity-specific obesity cut-points in the development of Type 2 diabetes - a prospective study including three ethnic groups in the United Kingdom. *Diabet Med* 2015; **32(2)**: 226-34.
  44. Wulan SN, Westerterp KR, Plasqui G. Metabolic profile before and after short-term overfeeding with a high-fat diet: a comparison between South Asian and White men. *Br J Nutr* 2014; **111(10)**: 1853-61.
  45. Wulan SN, Schrauwen-Hinderling VB, Westerterp KR, Plasqui G. Liver fat accumulation in response to overfeeding with a high-fat diet: a comparison between South Asian and Caucasian men. *Nutrition & Metabolism* 2015; **12(1)**: 1-9.
  46. Gillberg L, Perfilyev A, Brons C, Thomasen M, Grunnet LG, Volkov P *et al.* Adipose tissue transcriptomics and epigenomics in low birthweight men and controls: role of high-fat overfeeding. *Diabetologia* 2016; **59(4)**: 799-812.
  47. Galgani J, Ravussin E. Energy metabolism, fuel selection and body weight regulation. *Int J Obes (Lond)* 2008; **32(Suppl 7)**: S109-19.
  48. Lammert O, Grunnet N, Faber P, Bjornsbo KS, Dich J, Larsen LO *et al.* Effects of isoenergetic overfeeding of either carbohydrate or fat in young men. *Br J Nutr* 2000; **84(2)**: 233-45.

49. Sobrecases H, Le KA, Bortolotti M, Schneiter P, Ith M, Kreis R *et al.* Effects of short-term overfeeding with fructose, fat and fructose plus fat on plasma and hepatic lipids in healthy men. *Diabetes Metab* 2010; **36**(3): 244-6.
50. Lecoultre V, Egli L, Carrel G, Theytaz F, Kreis R, Schneiter P *et al.* Effects of fructose and glucose overfeeding on hepatic insulin sensitivity and intrahepatic lipids in healthy humans. *Obesity (Silver Spring)* 2013; **21**(4): 782-5.
51. Bray GA, Redman LM, de Jonge L, Rood J, Smith SR. Effect of three levels of dietary protein on metabolic phenotype of healthy individuals with 8 weeks of overfeeding. *J Clin Endocrinol Metab* 2016; **101**(7): 2836-43.
52. Rosqvist F, Iggman D, Kullberg J, Cedernaes J, Johansson HE, Larsson A *et al.* Overfeeding polyunsaturated and saturated fat causes distinct effects on liver and visceral fat accumulation in humans. *Diabetes* 2014; **63**(7): 2356-68.
53. Chiu S, Sievenpiper JL, de Souza RJ, Cozma AI, Mirrahimi A, Carleton AJ *et al.* Effect of fructose on markers of non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of controlled feeding trials. *Eur J Clin Nutr* 2014; **68**(4): 416-23.
54. Ma J, Karlsen MC, Chung M, Jacques PF, Saltzman E, Smith CE *et al.* Potential link between excess added sugar intake and ectopic fat: a systematic review of randomized controlled trials. *Nutr Rev* 2016; **74**(1): 18-32.
55. Chiavaroli L, de Souza RJ, Ha V, Cozma AI, Mirrahimi A, Wang DD *et al.* Effect of Fructose on Established Lipid Targets: A Systematic Review and Meta-Analysis of Controlled Feeding Trials. *J Am Heart Assoc* 2015; **4**(9): e001700.
56. Koopman KE, Caan MW, Nederveen AJ, Pels A, Ackermans MT, Fliers E *et al.* Hypercaloric diets with increased meal frequency, but not meal size, increase intrahepatic triglycerides: a randomized controlled trial. *Hepatology* 2014; **60**(2): 545-53.
57. Seyssel K, Alligier M, Meugnier E, Chanseaume E, Loizon E, Canto C *et al.* Regulation of Energy Metabolism and Mitochondrial Function in Skeletal Muscle during Lipid Overfeeding in Healthy Men. *J Clin Endocrinol Metab* 2014; **99**(7): 1254-62.
58. Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CWJ. Body-Mass Index and Mortality in a Prospective Cohort of U.S. Adults. *New England Journal of Medicine* 2008; **341**: 1097-1105.
59. Hamer M, Stamatakis E. Metabolically healthy obesity and risk of all-cause and cardiovascular disease mortality. *J Clin Endocrinol Metab* 2012; **97**(7): 2482-8.

60. Cornier MA, Von Kaenel SS, Bessesen DH, Tregellas JR. Effects of overfeeding on the neuronal response to visual food cues. *Am J Clin Nutr* 2007; **86**(4): 965-71.
61. Cornier MA, Salzberg AK, Endly DC, Bessesen DH, Rojas DC, Tregellas JR. The effects of overfeeding on the neuronal response to visual food cues in thin and reduced-obese individuals. *PLoS One* 2009; **4**(7): e6310.
62. Cornier MA, Grunwald GK, Johnson SL, Bessesen DH. Effects of short-term overfeeding on hunger, satiety, and energy intake in thin and reduced-obese individuals. *Appetite* 2004; **43**(3): 253-9.
63. Knudsen SH, Hansen LS, Pedersen M, Dejgaard T, Hansen J, Hall GV *et al.* Changes in insulin sensitivity precede changes in body composition during 14 days of step reduction combined with overfeeding in healthy young men. *Journal of applied physiology* 2012; **113**(1): 7-15.
64. Walhin JP, Richardson JD, Betts JA, Thompson D. Exercise counteracts the effects of short-term overfeeding and reduced physical activity independent of energy imbalance in healthy young men. *The Journal of physiology* 2013; **591**(Pt 24): 6231-43.
65. van der Meer, Hammer S, Lamb HJ, Frolich M, Diamant M, Rijzewijk LJ *et al.* Effects of short-term high-fat, high-energy diet on hepatic and myocardial triglyceride content in healthy men. *Journal Clin Endocrinol Metab* 2008; **93**(7): 2702-8.
66. Sevastianova K, Santos A, Kotronen A, Hakkarainen A, Makkonen J, Slander K *et al.* Effect of short-term carbohydrate overfeeding and long-term weight loss on liver fat in overweight humans. *Am J Clin Nutr* 2012; **96**(4): 727-34.
67. Boon MR, Bakker LE, Haks MC, Quinten E, Schaart G, Van Beek L *et al.* Short-term high-fat diet increases macrophage markers in skeletal muscle accompanied by impaired insulin signalling in healthy male subjects. *Clin Sci* 2015; **128**(2): 143-51.
68. Jebb SA, Siervo M, Fruhbeck G, Goldberg GR, Murgatroyd PR, Prentice AM. Variability of appetite control mechanisms in response to 9 weeks of progressive overfeeding in humans. *Int J Obes (London)* 2006; **30**(7): 1160-2.
69. Cahill F, Shea JL, Randell E, Vasdev S, Sun G. Serum peptide YY in response to short-term overfeeding in young men. *Am J Clin Nutr* 2011; **93**(4): 741-7.
70. Wadden D, Cahill F, Amini P, Randell E, Vasdev S, Yi Y *et al.* Serum acylated ghrelin concentrations in response to short-term overfeeding in normal weight, overweight, and obese men. *PLoS one* 2012; **7**(9): e45748.
71. Wadden D, Cahill F, Amini P, Randell E, Vasdev S, Yi Y *et al.* Circulating glucagon-like peptide-1 increases in response to short-term overfeeding in men. *Nutr metab (Lond)* 2013; **10**(1): 33.

72. Germain N, Galusca B, Caron-Dorval D, Martin JF, Pujos-Guillot, Boirie Y *et al.* Specific appetite, energetic and metabolomics responses to fat overfeeding in resistant-to-bodyweight-gain constitutional thinness. *Nutr Diabetes* 2014; **4**: e126.

## 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.*<sup>20</sup>)





Table 1

Reference	Baseline characteristics	Mean Age (y)	Mean BMI (kg/m <sup>2</sup> )	Overfeeding regime	Period	Activity	Body composition analysis modality
Van der Meer <i>et al.</i> 2008 <sup>65</sup>	15 healthy men	25±6.6	23.4±2.5	Normal diet + 2632 kcal/d; 94% F	3 days	Free living	Cardiac and liver <sup>1</sup> H-MRS
Tchoukalova <i>et al.</i> 2010 <sup>14</sup> and Votruba <i>et al.</i> <sup>34</sup>	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 <i>et al.</i> 2012 <sup>66</sup>	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 <sup>1</sup> H-MRS
Alligier <i>et al.</i> 2012,2013 <sup>26, 30</sup>	44 healthy men	33±1	NR (range 18-30)	Regular diet + 760kcal/d; 91% F	56 days	Usual	DEXA Abdominal MRI (T1-weighted)
Knudsen <i>et al.</i> 2012 <sup>63</sup>	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 <i>et al.</i> 2014 <sup>66</sup>	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 <sup>1</sup> H-MRS
Johannsen <i>et al.</i> 2014 <sup>63</sup>	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) <sup>1</sup> H-MRS of liver and soleus muscle
Rosqvist <i>et al.</i> 2014 <sup>52</sup>	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 <sup>1</sup> H-MRS liver Pancreatic MRS
Fabbrini <i>et al.</i> 2015 <sup>31</sup>	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 <sup>1</sup> H-MRS
Boon <i>et al.</i> 2015 <sup>67</sup>	24 healthy men	22.1±0.4	21.5±0.4	Regular diet +1275kcal/d; 94% F	5 days	No physical activity	Liver <sup>1</sup> H-MRS
McLaughlin <i>et al.</i> 2016 <sup>32</sup>	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 <sup>1</sup> H-MRS

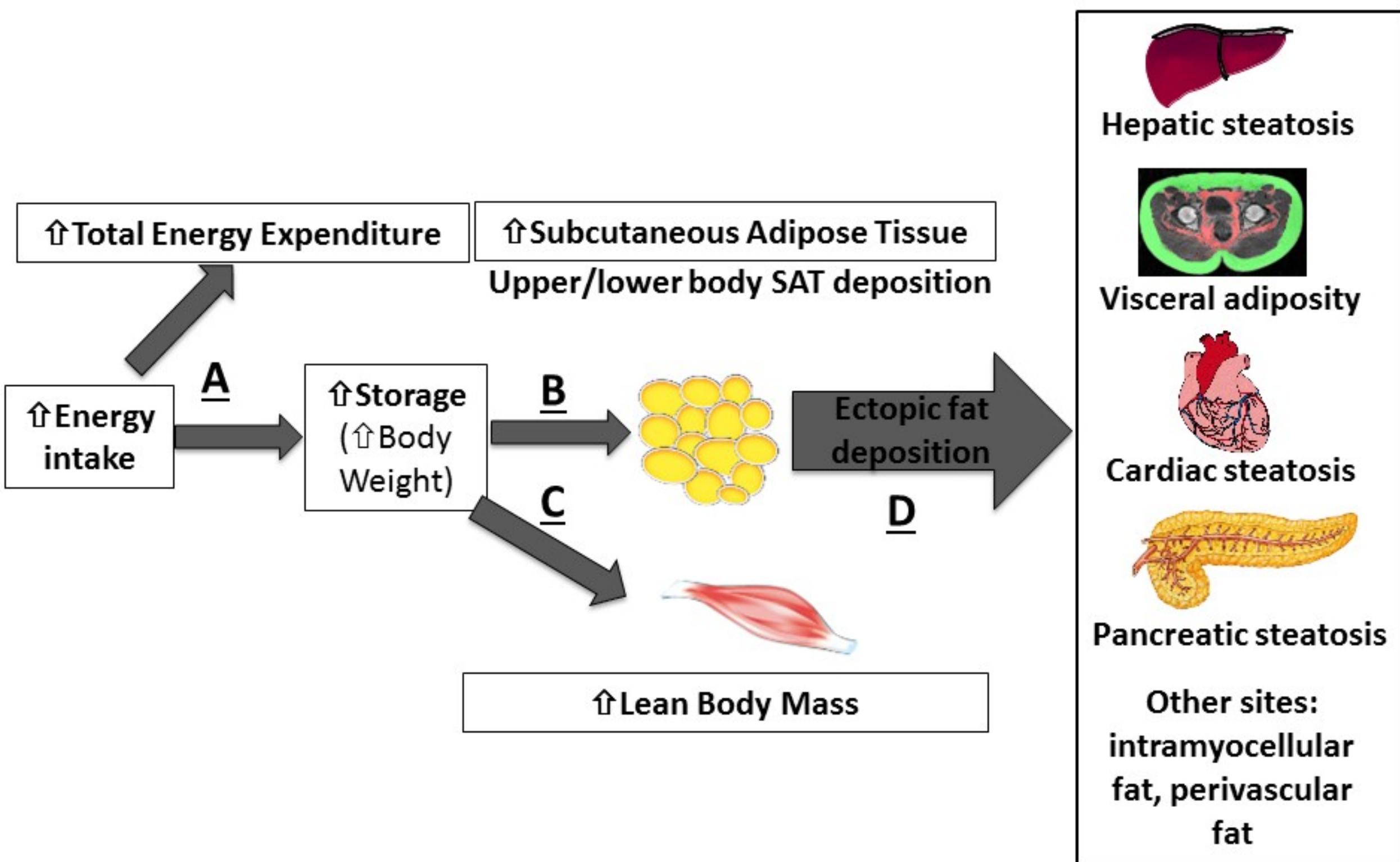
Table 2

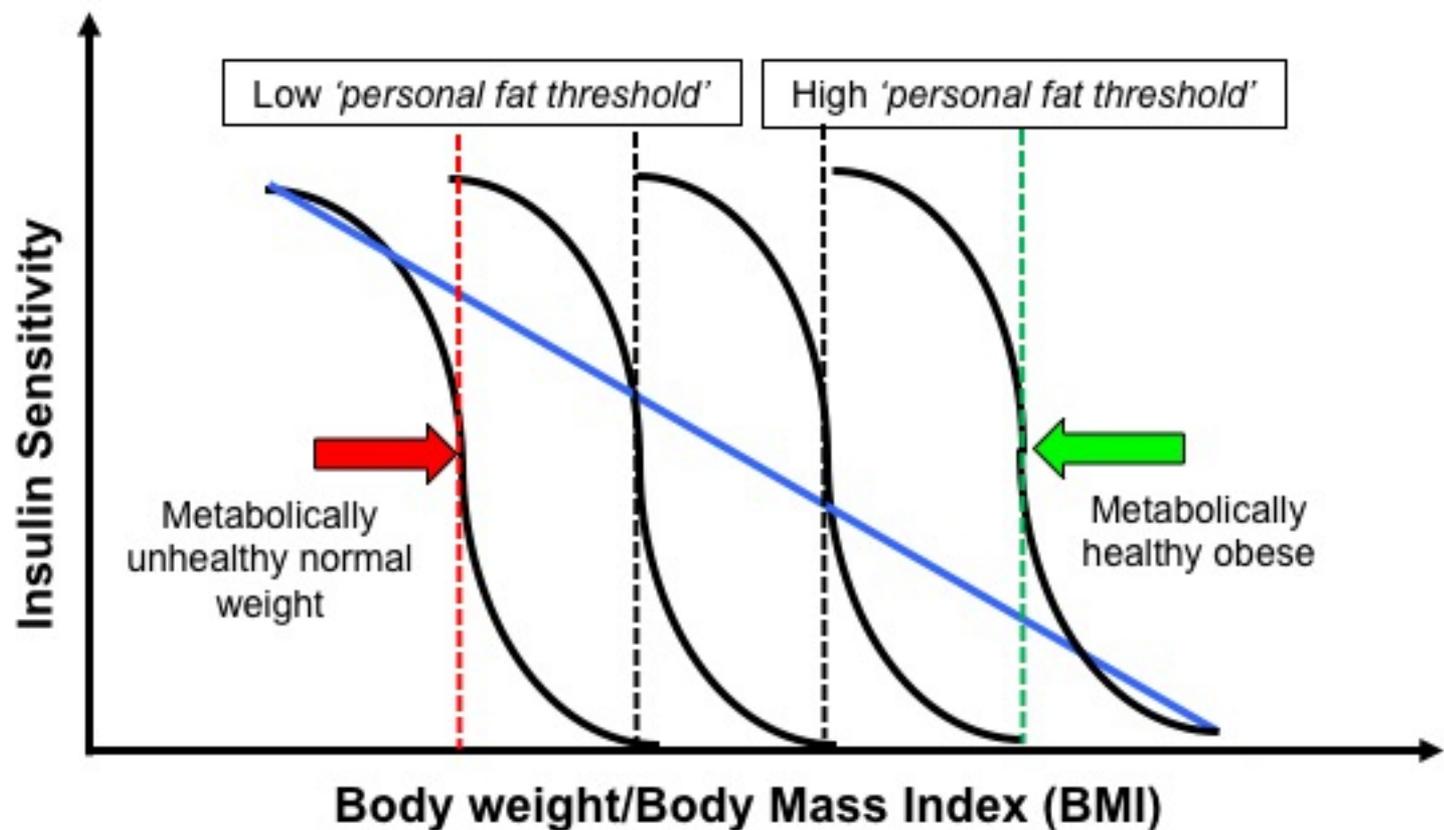
Reference	Weight gain (kg)	Changes in fat distribution			Adipocyte response	Metabolic response		Key findings
		Changes in SAT	Changes in VAT	Changes in liver fat		Insulin Sensitivity	Lipid levels	
Van der Meer <i>et al.</i> 2008 <sup>65</sup>	BMI increased 23.4±2.5 to 23.6±2.5 Change in weight not reported	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 <i>et al.</i> 2010 <sup>14</sup> and Votruba <i>et al.</i> 34	4.6±2.2kg	<b>Upper body:</b> +22.0±2.6% (women)  +41.0±7.3% (men) <b>Lower body:</b> +18.2±1.3% (women)  +34.9±5% (men)	+40.5±5.8	NA	<b>Femoral/abdo SAT Size (µg lipid/cell):</b>  Abdo: +39±11% Femoral: ±12±8% <b>No. (x10<sup>6</sup>):</b>  Upper body: +3±5%  Lower body: +23±7%	<b>24 Insulin AUC</b> 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 <i>et al.</i> , 2012 <sup>66</sup>	1.8±0.3kg  (88.7±4.1 to 90.5±4.1kg)	4440 (3700-6210) to 4570 (4000-6280)cm <sup>3</sup>	2180±300 to 2290±310cm <sup>3</sup>	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 <b>Lipogenic index</b> 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 <i>et al.</i> 2012,2013 <sup>26,30</sup>	2.5kg 79.1±1.8 to 81.6±1.8kg	91±7 to 100±7cm <sup>3</sup>	92±11 to 102±11cm <sup>3</sup>	NA	<b>Abdominal SAT Size (cell surface µm<sup>2</sup>)</b> 3123±129 to 3120±160 <b>Number (cells/mm<sup>2</sup>)</b> 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 <i>et al.</i> 2012 <sup>63</sup>	1.6kg  71.3±3.5 to 72.9±3.4kg	NA	28.8±13.5 to 43.1±20.5cm <sup>3</sup>	NA	NA	HOMA-IR 1.1 to 1.6  <b>OGTT AUC</b> increased 37±10%  <b>Clamp: glucose infusion rate</b> reduced by 43.6±11%.  <b>Matsuda index</b> reduced by 26±14%	TG 0.92 (0.64-1.3) to 1.13 (0.89-1.43) mM <b>FFA</b> 362.5(267.5-491.2) to 233.4 (138.5-393.1) µM	Reduction in insulin sensitivity precedes changes in body composition.
Koopman <i>et al.</i> 2014 <sup>66</sup>	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	IHTG: 0.83±0.38 to 1.00±0.78%  IHTG: 1.22±0.93 to 2.18±1.9%	NA	<b>Clamp:</b> 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 <i>et al.</i> 2014 <sup>33</sup>	+7.6±2.1kg  (81.9±10.3 to 89.5±9.4kg)	<b>Abdominal SAT:</b> +1.3kg (4.1±1.5 to 5.4±1.8kg)	<b>Abdominal VAT:</b> +0.36kg (0.58±0.49 to 0.94±0.58kg)	IHTG: 1.5±0.6 to 2.19±1% <b>IMCL:</b> 0.45±0.24% to 0.49±0.24%	NA	<b>Clamp</b> (glucose infusion rate): <b>Low dose insulin:</b> +18%  <b>High dose insulin:</b> +5% <b>EGP suppression:</b> 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 <i>et al.</i> 2014 <sup>62</sup>	PUFA 1.6±0.85kg (BL 67.4kg)  SFA 1.6±0.96kg (BL 63.3kg)	<b>Abdominal SAT:</b> 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 <i>et al.</i> 2015 <sup>31</sup>	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 <sup>3</sup>	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 <sup>3</sup>	MAO: +12%; 1714±585 to 1912±645cm <sup>3</sup>	MAO: 15.2±4 to 22.8±4.3%		MAO: +22% (baseline 6)  <b>Clamp:</b> Suppression of glucose rate of appearance lower in MAO group.	MAO +27% (134±61 to 170±52)  <b>VLDL apoB100:</b> secretion increased in MAO but not MNO (p=0.004)	in metabolically healthy but not in metabolically unhealthy
<b>Boon et al 2015</b> <sup>67</sup>	69.1±1.9 to 69.6±1.9kg	NA	NA	<b>IHTG:</b> 1.57±0.27% to 3.43±0.49%	NA	<b>HOMA-IR:</b> 1.62±0.26 to 2.39±0.32	<b>TG (mmol/l):</b> 1.0±0.1 to 1.0±0.1 <b>NEFA (mmol/l)</b> 0.5±0.03 to 0.5±0.03	NA
<b>McLaughlin et al 2016</b> <sup>32</sup>	<b>IS</b> 86.2±10.1 to 89.6±10.3kg  <b>IR</b> 89.4±11.2 to 92.1±11.1kg	<b>IS:</b> 147 ± 54 to 162 ± 51cm <sup>3</sup>  <b>IR:</b> 140 ± 34 to 148 ± 37cm <sup>3</sup>	<b>IS:</b> 37±22 to 44±28cm <sup>3</sup>  <b>IR:</b> 64±16 to 73±27cm <sup>3</sup>	<b>IHTG: IS:</b> 0.03 ± 0.21 to 0.07 ± 0.04  <b>IHTG: IR:</b> 0.23±0.31 to 0.3±0.22	<b>Abdominal SAT size and structure:</b>  Peak adipocyte diameter increased significantly only in IS subgroup.  Significant decrease in percentage of small adipose cells in IS	<b>Muscle insulin resistance</b> worsened in IS group only: 45%(IS) vs. 8%(IR)	<b>Insulin suppression of lipolysis</b> 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 <sup>2</sup> )	Dietary protocol	Period	Activity	Weight gain	Changes in appetite	Changes in gut hormones
Cornier et al, 2004 <sup>62</sup>	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. <b>Ad libitum energy intake:</b> following OF non-significantly reduced in all.	N/A
Jebb et al, 2006 <sup>68</sup>	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	<b>Food intake</b> stimulated overall during free diet period. Variable change with 'compensators' and 'non-compensators'.	<b>Leptin</b> elevated (+116%)
Cornier et al, 2007 <sup>61</sup>	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 ( <i>high hedonic value</i> > <i>neutral hedonic value</i> ) blunted by overfeeding.  VAS: reduced hunger and increased satiety ratings.	N/A
Cahill et al., 2011 <sup>69</sup>	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	<b>Serum PYY</b> concentration significantly increased in response to overfeeding
Wadden et al., 2012 <sup>70</sup>	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	<b>Fasting serum acylated ghrelin</b> increased in all groups in response to overfeeding
Wadden et al., 2013 <sup>71</sup>	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	<b>Fasting GLP-1</b> increased in all groups with no difference based on weight status
Germain et al., 2014 <sup>72</sup>	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	<b>Incremental AUC for PYY and GLP-1</b> 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 <sup>73</sup>	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	<b>Ad libitum intake</b> 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





— Epidemiological hypothesis of linear relationship between BMI and insulin sensitivity

- - - Individual's 'personal fat threshold'