Efficiency of auto-regulatory homeostatic responses to imposed caloric excess in lean men.

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Running Head: Autoregulatory responses to caloric excess

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ABSTRACT

Obesity implies a failure of auto-regulatory homeostatic responses to caloric excess. We studied the mechanisms, effectiveness and limits of such responses in 6 lean (21.9±1.3 kg/m²), healthy men based in a metabolic suite for 17 weeks progressive intermittent overfeeding (OF) (3wk baseline, 3wk 20%OF, 1wk ad lib, 3wk 40%OF, 1wk ad lib, 3wk 60%OF, 3wk ad lib). Body composition was assessed by 4-compartment model using dual X-ray absorptiometry, deuterium dilution and plethysmography. Magnetic resonance imaging assessed subcutaneous/visceral fat at abdominal level at baseline and end of 60%OF. Energy intake was assessed throughout, energy expenditure (calEE) and substrate oxidation rates were measured repeatedly by whole body calorimetry, and free-living energy expenditure (TEE) by doubly labelled water (DLW) at baseline and after 60%OF. At the end of 60%OF, calEE and TEE had increased by just 11.4 (p=0.001) and 16.2% (p=0.001), respectively. Weight and body fat (FM) had increased by 5.98kg (8.8%, p=0.001) and 3.31kg (22.6%, p=0.01). The relative increase in visceral fat (32.6%, p=0.02) exceeded subcutaneous fat (13.3%, p=0.002) in the abdominal region. The computed energy cost of tissue accretion differed from the excess ingested by only 13.1% (using calEE) and 11.6% (using TEE) indicating an absence of effective dissipative mechanisms. We conclude that elevations in energy expenditure provide very limited auto-regulatory capacity in body weight regulation, and that regulation must be dominated by hypothalamic modulation of energy intake. This result supports current conclusions from genetic studies in which all known causes of human obesity are related to defects in the regulation of appetite.

Key words: energy balance, body composition, whole-body calorimetry, doubly labelled water, overfeeding, dissipative mechanisms
INTRODUCTION

Overeating, whether chronic or episodic, produces a positive energy balance and favors the accretion of new tissues, particularly fat (35, 49). The efficiency of innate auto-regulatory mechanisms that attempt to maintain body weight homeostasis has been explored extensively during the last century since the earliest experiments published by Neumann in 1902(30) and Gulick in 1922(14). These suggested the existence of energy dissipative mechanisms (termed *luxuskonsumption*) able to dispose of part of the energy excess as heat and decrease the storage of energy as fat. These two overfeeding studies were conducted on only one subject and the authors claimed that an increase in energy expenditure dissipated the extra energy available and prevented weight gain. Forbes (11) reexamined the relationship between weight gain and excess energy intake in these two studies and observed that the slope of the regression line between weight gain and excess energy intake was close to the predicted cost of weight gain. Since then numerous studies performed under experimental (3, 8, 17, 22, 24-26, 37, 41) and non-experimental settings (5, 28, 33, 45) have failed to reach a consensus (40, 47, 51, 53). The controversy was mainly focused on the identification of the mechanisms disposing of the excess energy (futile cycles, non exercise activity thermogenesis, mitochondrial uncoupling proteins) and on the partitioning and storage of the available energy (fat mass, glycogen, lean mass). The study of such energy dissipating mechanisms is a methodological challenge as they are extremely sensitive to external confounding by various environmental influences (e.g. dieting, physical activity, eating behaviour, psychological and physical well-being, drugs)(20) and because measurement techniques of body composition have lacked sufficient precision. The most accurate and replicable conditions to study energy balance in humans require highly controlled experimental protocols where the manipulation of energy intake and macronutrient composition can be made with standardisation, and in
which precise measurement of physical activity level, energy metabolism and body composition is possible.

Many previous studies have employed very short-term protocols to probe possible metabolic responses, but these would not detect slowly-inducible mechanisms. Most of the previous longer-term studies have used a single (generally severe) level of overfeeding. Such conditions may overwhelm the available homeostatic processes and obscure more subtle changes that may be effective in modulating energy balance during naturally-occurring episodic periods of marginal excess consumption. To overcome these limitations we used state-of-the-art measurement methods in a highly-standardized 17 week protocol involving progressive overfeeding from 20% to 60% energy excess. Lean, healthy men were challenged with three 3 week periods of stepwise overfeeding (OF) separated by 1 week ad libitum energy intake. Our objectives were: 1) to study the limits of any putative energy-dissipating auto-regulatory mechanisms (the luxuskonsumption hypothesis); 2) to assess their importance relative to alterations in appetite and food intake during the subsequent ad libitum periods; and 3) to assess the induced changes in whole body and segmental body composition, in particular the balance between abdominal subcutaneous and visceral fat, to describe patterns of fat accretion during medium-term caloric excess.

**METHODS**

Changes in energy metabolism and body composition induced by experimental overfeeding were assessed in six healthy, weight-stable, habitually lean men. All subjects lived and worked in the Cambridge area and were recruited through the MRC Dunn Nutrition Unit’s register of volunteers. The study was approved by the Unit’s Ethics Committee. Subjects gave their written consent to participate in this study. The study was conducted at the former MRC Dunn Clinical Nutrition Centre (DCNC).
The selection criteria were good health, weight stability, habitual alcohol consumption <21 units/week, not vegetarian, non-smokers, no food intolerances and willingness to complete the study procedures. Throughout the study, subjects were provided with food, accommodation and a small honorarium. The subjects lived for the entire period in the DCNC metabolic facility and were allowed to leave only for short periods of time. The volunteers were instructed to maintain their usual level of physical activity and, except for the exercise performed in the metabolic chamber, deliberate additional exercise was not allowed. Medical conditions and potential side effects were regularly monitored and any health problem was reported and assessed by a medically qualified researcher. No subjects were excluded from the study for intercurrent adverse events. One subject did not complete the final OF period and ad libitum phase.

The study started with a baseline period of three weeks when the dietary intake provided was adjusted to maintain body weight. Subjects were then challenged with three-weeks stepwise overfeeding phases (+20%, +40%, +60% increases above the baseline energy intakes) separated by intermittent ad-libitum phases.

Three meals per day (breakfast, lunch, supper) were provided on a 4-d rotating menu. Meals were prepared in the DCNC metabolic kitchen. The diets were carefully designed both to include foods eaten in the UK and to avoid excess palatability. The calculation of the energy content of the diet was based on UK Food Composition tables (19). As OF progressed, the portion size of meals increased, and snacks were introduced to increase total EI. Subjects were required to consume all the food provided. During the intercurrent ad-libitum periods subjects ate the same pre-weighed diets provided at similar levels of excess as in the preceding overfeeding periods. All uneaten food was measured and total intake calculated. Water, tea or coffee (decaffeinated) were freely available.
During the baseline period subjects received a diet designed to meet their energy requirements (calculated as 1.5 x predicted basal metabolic rate), comprising 13% energy from protein (P), 40% from fat (F) and 47% from carbohydrate (C) and the amount of energy provided then adjusted to maintain weight stability. The subjects then received a fixed diet providing +20% (P = 13%; F = 43%; C = 44%) of the baseline intake for three weeks. Half the increase was achieved by an increase in portion size and the remainder by an increase in the proportion of fat. This was followed by a week of *ad libitum* food consumption in which subjects continued to be offered +20% of their baseline energy intake, but were allowed to eat only as much as desired. In week 6 the subjects progressed to the second stage of OF, comprising +40% (P = 12%; F = 46%; C = 42%) of baseline energy intake for three weeks followed again by a week of standardized *ad libitum* consumption with the same +40% diet offered. The final step of OF comprised a three-week period of +60% (P = 12%; F = 48; C = 40%) of baseline energy intake followed by three weeks *ad libitum* energy intake from the +60% diet. The energy density of the diets (MJ/100g) increased by only 10% between the baseline and +60%OF. Energy intake, energy density and nutrient content of the diets and snacks provided during baseline and OF are shown in Table 1. No assessment of metabolizable energy was performed because in previous analogous experiments we have found excellent agreement between metabolizable energy directly measured by bomb calorimetry of diets, feces and urine and calculated from food composition tables (r=0.99) as described elsewhere (8).

**Body Composition**

Body weight (± 10g) was measured weekly after voiding and before breakfast using a digital integrating scale (Sauter E1210, Suffolk, UK). Height was measured to the nearest 5 mm using a wall-mounted stadiometer (Holtain Ltd, Dyfed, Wales, UK) at the beginning of the study. Body mass index (BMI) was calculated as weight (kg)/height\(^2\) (m). Body
composition measurements were performed at the end of baseline, each overfeeding phase (20%OF, 40%OF, 60%OF) and at the end of the last *ad libitum* phase (AL3).

*Air-displacement plethysmography (ADP)*: Measurements were performed in duplicate using an air displacement plethysmograph (BODPOD, Life Measurement Instruments, Concord, CA) according to manufacturer's instructions. Thoracic gas volume was predicted by estimation of functional residual capacity (FRC) and tidal volume (Vt) (27). Siri’s two-compartment formula was used to calculate percentage fat mass (FM%) from body density (46). From FM% and body weight, fat mass (FM) and fat-free mass (FFM) in kilograms were calculated.

*Total body water (TBW)*: Total body water was measured by isotope dilution. After collection of a pre-dose saliva sample, subjects received an oral dose of deuterium oxide (0.7g/kg body weight) and saliva samples were collected at 4, 5 and 6h after the dose. The subjects refrained from eating or drinking 30-min before taking a saliva sample. The concentration of deuterium in each sample was measured using isotope ratio mass spectrometry as described elsewhere (18) and the pool size calculated. The measured pool size was reduced by 4% to account for the exchange of deuterium with non-aqueous hydrogen. The hydration fraction of FFM was assumed to be 0.7194 and FM was calculated as the difference between FFM and body weight. This method was used to measure TBW at the end of 20%OF, 40% OF and last *ad libitum* phase. Total body water was measured using the doubly labelled water protocol at the end of baseline and 60% OF. The influence of repeated doses of deuterium on the background level of deuterium enrichment and the effects on the measurement of TEE have been taken into account and corrected for as described elsewhere(38, 39).

*Dual energy X-ray absorptiometry (DXA)*: Whole-body DXA scans were performed using a Hologic QDR-1000W scanner (Hologic Inc., Waltham, MA, USA) and analysed
using an enhanced version of the software to estimate bone mineral mass (subsequently used to derive ‘ash’), bone mineral content (BMC), FM and FFM. Subjects were measured wearing a standard light cotton gown to minimize clothing absorption.

Total body scanning area was divided into anatomic segments: the arms were separated from the trunk by a line passing through the humeral head and the apex of the axilla. The trunk was separated from the legs by a line passing from the iliac crest to the perineum. The head was excluded from the trunk by a horizontal line passing just below the mandible.

Four-compartment model (4-C): The 4-C model divides the body into fat, water, protein, and mineral, thereby avoiding the assumption that the ratio between mineral and protein in FFM is constant. The body composition data were combined to yield an estimation of fat mass. FM (kg) = (2.747 x BV) + (0.710 x TBW) + (1.460 x BMC) + (2.050 x BW) where BV is body volume in litres, TBW is total body water (kg), BMC is bone mineral content (in kg) and BW is body weight (kg) (31). The protein plus carbohydrate (P+C) compartment was derived by difference (P+C = BW – TBW – TMM – FM). The precision of the three and four-compartment model to assess body fat was ± 0.49 kg and ± 0.54 kg respectively when a 1% precision for water estimation was assumed. The precision for estimates of TBW was based on sequential measurements of the isotopic enrichment of water in saliva samples taken at 4, 5 and 6h after oral administration of the isotope. Precision for the measurement of water calculated from this study was 0.45 kg (about 1%)(13).

Magnetic Resonance Imaging (MRI): Abdominal adipose tissue distribution was assessed by T1-weighted MRI with a single cross-sectional image at the level of the umbilicus. The area of the cross section of the torso (TCSA) was measured manually using an electronic cursor on the MRI work station (Advantage Windows, GE, Milwaukee, USA) as a region of interest. Then the cross sectional area of the intra-abdominal cavity was measured (IAC) just
internal to the rectus abdominis, trasversus muscle, iliacus muscle and aortic biforcation. It was assumed that any change in these areas was due to an alteration of fat. Visceral abdominal adipose tissue (VAT) was assumed to be equivalent to IAC. Subcutaneous abdominal adipose tissue (SAT) was calculated as the difference between the total cross sectional area (TCSA) and IAC.

Energy expenditure

Twenty-four hour whole-body indirect calorimetry measurements (24-hr EE) were performed at the end of each phase (baseline, overfeeding phases, ad libitum phases). Free-living total energy expenditure, using DLW, was measured only at baseline and during the last overfeeding phase (+60%).

Whole-body indirect calorimetry: The calorimeter chambers were comfortably furnished with a divan bed, armchair and entertainment facilities (TV, radio, telephone). All urine samples were collected for analysis. While in the calorimeter, subjects followed an identical protocol on each occasion with sleep, rest, meals and exercise (cycle-ergometer and stepping). Subjects entered the chamber at 20:00 h on day 0. A total of 37 h was spent inside, ending at 09:00 h on day 2. Exchange rates for oxygen and carbon dioxide were calculated using the expressions of Brown et al. (4) for pressure-ventilated systems. A detailed description of the calorimeters is given elsewhere (29). Briefly, measures of total energy expenditure (24-hr EE), basal metabolic rate (BMR) and activity plus thermogenesis (A+T) were obtained. BMR was measured for 1h, immediately on waking, between 12.5 and 13.5 hr post-absorption, at thermoneutrality, and at complete physical rest and was measured twice during each 37-hr period. Two 30-min periods of weight-dependent exercise (10 stepups per min on a 20-cm block), and two 30-min periods of weight-independent exercise (cycle ergometer at 25 W) were performed and the exercise periods were closely supervised. The value for activity plus thermogenesis (A+T) was calculated by subtracting BMR from 24-hr EE (24-hr EE minus
BMR). Calculation of macronutrient balance took into account the measurements made in the calorimeter at the end of each phase. Carbohydrate (CHO) and fat oxidation were calculated according to the method of Elia & Livesey (10), assuming an energy content of 39.33 kJ/g for fat, 15.76 kJ/g for CHO, and 18.56 kJ/g for urinary protein.

*Doubly labelled water (DLW):* Total energy expenditure (TEE) under free-living conditions was assessed by DLW during baseline and 60 % OF. Doses of deuterium (0.07 g/kg) and $^{18}$O (0.174 g/kg) were given orally 14 days before the end of each period. After collection of a pre-dose urine sample, samples were collected on the dosing day and daily for 14 days thereafter. Theoretical considerations concerning analysis, propagation of error and calculations of energy expenditure have been described elsewhere (6, 36). Isotope enrichment of the urine samples were analysed using continuous flow isotope ratio mass spectrometry (Sira 10 Dual Inlet Mass Spectrometer, Micromass, UK).

*Cost of weight gain:* The calculation of changes in energy stores and the agreement with excess energy intake was calculated according to Tremblay et al (48). The calculations for each OF phase are based on the body composition (4-C model) and 24-hr EE measurements in the calorimeter at the end of each OF phase. The calculations for the whole study used the body composition measurements (4-C model) and free-living TEE (DLW) performed at baseline and at the end of the 60% OF. The preceding *ad libitum* phases have been included in the calculations and assume that body composition and energy expenditure changes from the start of each *ad libitum* phase to the end of OF were linear. The calculated intrinsic energetic costs associated with tissue accretion (fat, protein) were added to the increase in TEE which includes any increase in thermic effect of food and energetic cost of physical activity associated with weight gain. The energy deposited as fat and protein was assumed to be 38.9 MJ/kg and 23.5 MJ/kg, respectively. The synthesis costs of fat and protein
have already been accounted for in the measurement of TEE. Possible changes in glycogen stores were ignored.

**Statistics**

Data are expressed as mean±s.d or mean±s.e. The absolute (x-y) and relative ([(x-y)/y]×100) changes from baseline were calculated for each variable (weight, body composition, energy intake, energy expenditure). Analysis of variance (ANOVA) for repeated measures and post-hoc analysis (Least Square Method) were used to detect significant changes between phases. Pearson correlation coefficient was used to test associations between variables. Commercial software packages (SPSS 14, SPSS, Inc, USA and Sigmaplot 10, Systat Software, Inc) were used. The significance level was set at p<0.05.

**RESULTS**

The characteristics of the 6 subjects are shown in Table 2. Mean age was 43.3 ± 10.6 years (range: 32 – 58y) and baseline values for weight, height and BMI were respectively 68.9 ± 8.8 kg (range: 56.0 – 80.8 kg), 1.77 ± 0.07 m (range: 1.63 – 1.83 m) and 21.9 ± 1.3 kg/m² (range: 20.8 – 24.1 kg/m²).

**Weight change**

Table 2 also shows individual and mean changes in body weight and fat mass throughout the study. The periods of imposed energy excess led to a consistent weight gain, but weight changes were more variable during the *ad-libitum* phases. Three subjects gained weight during the first and second *ad-libitum* phase and three subjects lost weight. Body weight decreased in all subjects during the last *ad libitum* phase. During the 20%OF there was a cumulative weight gain of 0.70kg (ns), which was substantially unchanged (+0.73kg vs baseline) by the end of the subsequent *ad-libitum* phase. Weight continued to increase during the 40%OF (+2.54 kg vs baseline, p=0.001) followed by a loss of 0.44kg by the end of the second *ad-libitum* phase. Weight gain reached its peak at the end of the 60%OF (+5.98kg vs
baseline, p=0.001); a cumulative weight increase of 8.8% from baseline. Despite a weight loss of 2.71kg during the last *ad-libitum* phase, subjects gained a net 3.27kg (5%, p=0.03) by the end of the study, ranging from 0.93 to 5.83kg (1.4 to 9.8%).

Analysis of the weekly rate of weight change highlighted the responses to overeating and *ad libitum* intake. The rate of weight gain during OF phases was greater than the rate of weight loss achieved during the subsequent *ad-libitum* phases. Weight continued to increase during the first *ad-libitum* phase (0.03kg/week) and the weight gain during the 40%OF (0.73kg/week) was greater than the weight decrease during the second *ad-libitum* phase (-0.44kg/week). The rate of weight gain during the 60%OF (1.12kg/week) was almost counterbalanced (~0.90kg/week) during the final *ad-libitum* phase.

**Body composition**

Changes in body composition are shown in Figures 1 and 2. The change in body fat (FM) was proportional to the degree of OF and, by the end of the 60%OF phase, there was a significant increase in FM (3.31kg, p=0.01), followed by a decrease of 1.61kg during the last *ad-libitum* phase. Fat free mass also increased by 2.67kg (p=0.07) by the end of 60%OF. Most of the change in FFM was due to changes in TBW, which explained, on average, 87% of the FFM gain and 86% of FFM loss during the OF and *ad-libitum* phases, respectively. The contribution of protein to FFM change increased during the first two OF phases (20%OF = 21%; 40%OF = 39%) but declined to 8% during the 60%OF. There were no significant changes in total mineral content (TMC).

The exclusion of the first subject from the analysis did not significantly alter the results. In the remaining five subjects, the segmental body composition analysis using DXA showed that truncal fat mass increased by approximately 60% at the peak of weight gain compared to only 28% and 17% in upper and lower limbs (Figure 2). The substantial increase in truncal fat mass was confirmed by MRI. At the end of the +60 %OF, the relative increase
in visceral fat was 32.6% (38.6cm$^2$, p=0.02) and the increase in subcutaneous fat 13.3% (47.7cm$^2$, p=0.002) (Figure 2).

**Energy expenditure**

Table 3 shows BMR and 24-hr EE measured with whole-body calorimeter (CalEE) and TEE measured by doubly labelled water.

**Whole-body indirect calorimetry:** On average, CalEE increased during the OF phases and decreased during the *ad libitum* phases although it remained above the baseline values. CalEE at the end of 20%, 40% and 60% OF was 11.7, 11.9 and 12.7MJ/day and the cumulative difference relative to baseline was statistically significant during each OF phase: 20% (+0.39MJ/day; p=0.03), 40% (+0.51MJ/day; p=0.001) and 60% (+1.32MJ/day; p=0.001). BMR increased significantly during the 40% (+0.39MJ/day; p=0.02) and 60%OF (+0.96MJ/day; p=0.007). The energy expended in activity plus thermogenesis (A+T) showed a significant increase during 60% OF (8.2%), reflecting increased thermogenesis, followed by a decrease during the last *ad libitum* phase (-6.4% vs baseline) when subjects were all in negative energy balance (data not shown). Baseline calEE/FFM and BMR/FFM were 209.1kJ/kgFFM and 127.6kJ/kgFFM, respectively. At the end of 60%OF, calEE/FFM was increased by 6% (ns) and BMR/FFM by 8% (ns). The absolute changes in calEE measured at the end of each OF phase ($\Delta$ calEE) were significantly associated with $\Delta$ FM (r = 0.53; p=0.02) and $\Delta$ weight (r = 0.72; p=0.001) but surprisingly not with $\Delta$ FFM (r = 0.30; ns). At the end of 60% OF an indirect association was observed between percent change ($\Delta\%$) in calEE and $\Delta\%$ body weight (n=5; r= - 0.71; p=0.17) indicating that subjects with a greater increase in calEE experienced a lower weight gain.

**Doubly labelled water (DLW):** There was a significant increase (17%) in TEE at the end of 60%OF relative to baseline (+1.89MJ/day; p=0.01). The physical activity level
(PAL=TEE/BMR) was not significantly different from baseline (PAL_{Bas} = 1.60 vs PAL_{60\%OF} = 1.65; ns).

**Energy intake**

On average, there was an absence of compensatory adjustments to OF. The mean energy intake (EI) during *ad libitum* periods was not significantly different from baseline for all the *ad libitum* phases as a result of the large between-subject variability. Indeed, during the first and second *ad libitum* periods EI was +4.9 % and +10% higher than baseline. During the final *ad libitum* period, EI was decreased compared with the preceding OF period (-38%) and comparable to baseline (-2.3%) (Figure 3).

**Macronutrient Oxidation**

Macronutrient balance calculated as macronutrient intake (MJ/day) minus substrate oxidation (MJ/day), at baseline and at the end of each OF is shown in Figure 4. The analysis was carried out on the five subjects who completed all phases to allow direct comparisons. Stepwise OF promoted fat accumulation by reciprocal changes in fat (decrease, p=0.01) and carbohydrate oxidation (increase, p=0.001). There was a significant increase in protein oxidation (p=0.007) although the contribution to overall energy balance was minimal. The net change relative to baseline at the end of 60%OF was +0.4MJ/day for protein, -1.3MJ/day for fat and +2.1MJ/day for carbohydrate oxidation.

At the end of baseline subjects were in fat balance (ns) but by the end of the +20%OF fat intake significantly exceeded oxidation (p=0.003) and the gap became progressively larger in subsequent OF phases (+40% OF: p=0.003; +60% OF: p=0.001), which was, as expected, associated with weight gain (r = 0.83, p=0.001). It is notable that there was no detectable net change in carbohydrate balance. Despite an increase in protein oxidation, this was consistently lower than protein intake and subjects were in positive nitrogen balance.
Cost of weight gain

The calculations of the cost of weight gain are shown in Table 4. The first phase (OF +20%CAL) showed a high discrepancy between energy surplus and cost of weight gain (26.98 MJ) and 71% of the energy was unaccounted for by changes in energy stores and calEE. However, the mean change in body composition at this stage was very small (FM= +0.08kg) with substantial inter-individual variability, with measurements in two subjects indicating opposite changes in fat mass (loss) and total body water (gain).

The energy cost of fat and protein deposition and increased calEE explains almost all the extra energy consumed during the 60%OF (13.1%). The measurement of calEE in calorimeters does not reproduce free-living conditions and, if this is increased by 25% as suggested by Ravussin et al (37), the unexplained energy is only 9.3% for the 60%OF.

The use of TEE measured in free-living conditions using doubly labelled water allows a better estimation of the changes in energy expenditure. Using this method there is near perfect agreement between the total average energy excess throughout the study (238.9 MJ) and the total energy explained by tissue accretion and increased TEE (211.16 MJ) based on the assumption of a linear increment in energy expenditure between baseline and the 60%OF measurement. The unexplained energy was only 27.7 MJ (11.6%). The average energy equivalent of weight gain calculated for the whole study, assuming a linear increase in TEE, was 19.25 MJ/kg. The theoretical calculation of the cost of weight gain based on average FM and FFM changes and assuming a protein content of 20.6% for FFM was calculated as (38.9 x 0.37) + (23.6 x 0.20 x 0.62) = 17.3 MJ/kg. The observed mean value of cost of weight gain differed from this by only 9%.
DISCUSSION

In everyday life, overeating occurs spontaneously in many individuals in response to environmental, psychological and social cues (16). In experimental settings the replication of free-living conditions to investigate human energy metabolism is problematic but this study has attempted to reproduce the recurrent periods of overeating that characterize the weight history of the vast majority of overweight subjects (9). To our knowledge this represents the first study of this kind as previous overfeeding studies are characterized by a continuous overfeeding rather than stepwise increases in energy intake separated by *ad libitum* phases. This novel paradigm allows the measurement of both changes in EE and compensatory effects on appetite control.

The net result of this imposed overfeeding regimen was a weight gain of 6 kg by the end of the 60%OF. Fat gain accounted for 55% of the increase and fat free mass gain for 45%. This ratio is comparable to the study of Ravussin et al (37) when five subjects were overfed (+60%) for 9 days (FM = 57%; FFM = 43 %). Diaz et al. (8) overfed nine subjects for 42 days by 50% above energy requirements; subjects gained 7.6 ± 1.6 kg of body weight and FM accounted for 58% of the change in body weight. This study showed that changes in body composition during the OF phases were in close agreement with the theoretical calculations proposed by Forbes et al.(11), which provided a detailed analysis of the relationship between cost of weight gain and overfeeding in experimental conditions. They observed that overfeeding always induced weight gain in experimental conditions; that weight gain is proportional to the total amount of energy excess consumed and that the average composition of weight gain was 44% lean and 56% fat, which are nearly identical to the changes in body composition observed in this study. Total body water explained most of the variation in the change in body mass initially but its contribution declined when energy intake and fat accretion increased. The preferential deposition or mobilization of glycogen stores in response
to initial changes would cause an initial, much larger displacement of glycogen than fat stores, which might explain the initial, higher shifts in total body water (15, 44). Previous overfeeding studies have not used multi-compartment models to measure body composition changes and a direct comparison with our results is not possible.

A striking observation was the high between-subject variability in weight change during the *ad libitum* phases which reflects different compensatory responses to OF. Some subjects were able to control their EI (compensators) but others were not (non-compensators) perhaps indicating an interaction between physiological and cognitive mechanisms, the latter arising from the perception of an increased body weight and/or food portions (8, 23, 31). The inability of subjects to return to their baseline levels of energy intake points to an asymmetric regulation of appetite in humans as has been previously noted (1, 23). Diaz et al. (8) did not show data on energy intake during their 6 weeks *ad libitum* post-overfeeding phase. However, body weight did not return to baseline values and subjects were able to lose only 55% of the body weight gained. The variability in weight loss was significant, ranging from 42% to 86% and probably reflected an individual ability to reduce energy intake between subjects and compensate for the preceding overfeeding. The compensation for overeating was also explored in 12 pairs of monozygotic twins after 4 months from the end of an 84-day overfeeding study (Quebec Twin Study) and, in free-living conditions and without controlling for physical exercise, the average body weight was still above baseline by 1.4 kg(48). The same study re-measured body weight five years later to explore inter-individual variability of weight change and uncover individual weight trajectories (2).

Intra-pair trajectories of weight gain were collinear, whereas the inter-pair trajectories were divergent and associated with variable rates and amounts of weight change during both overfeeding and free-living periods (3, 7, 48, 50). It was evident that genotype influences the metabolic responses (energy expenditure, body composition) to the imposed energy excess in
standardized and non-standardized conditions and contributes significantly to the between-subject variability.

Overfeeding in our study was associated with marked changes in visceral fat in this group of lean subjects. At the end of 60% OF the relative increase in visceral fat was nearly twice that of subcutaneous fat, which confirms the more active energy mobilization and deposition of visceral adipocytes (12) and this demonstrates that even short bouts of overeating may induce greater metabolic effects which may initiate some of the mechanisms leading to insulin resistance (43). In contrast CT scans in the Quebec Twin overfeeding study showed a higher proportion of fat deposited as subcutaneous abdominal tissue which increased by 95% while the visceral layer increased by 70% (3). The greater proportion of visceral fat gain in our shorter study might have two possible explanations that need not be mutually exclusive: either that the visceral depot acts as a short-term ‘buffer’ that can rapidly assimilate fat prior to a later redistribution; or that the visceral depot has a relatively limited capacity and once this is reached fat is then preferentially diverted to subcutaneous regions. The dynamics of such differential storage would be complex given that fat loading will induce the formation of new adipocytes that will gradually increase depot capacity.

As expected, the imposed positive energy balance was associated with a progressive increase in calEE and BMR by 11.4 and 14.4%, respectively. The increase BMR explained more than 70% of the total change in calEE and more than 55% of the total change in free-living TEE. These figures are close to the increases in calEE seen in previous overfeeding studies. Diaz et al.(8) observed an average rise in calEE of 17% and about 50% was due to an increase in BMR. Webb et al.(52) overfed eight subjects for 30 days and they observed an increase in TEE by 7.4%. The Twin Overfeeding Study overfed 24 twins by a total of 353 MJ which produced an increase in BMR by 9.6% and accounted for 46% of the total change in TEE(48). Conversely, during the ad libitum phases there was a decrease in BMR and calEE;
however, BMR was still above baseline values because weight was higher at the end of the last *ad libitum* phase whereas calEE was similar to baseline values due to the drop in A+T.

The ability of the body to accumulate fat when in a positive energy balance and prioritise carbohydrate oxidation over protein and fat is an established mechanism that we have previously described in terms of an ‘oxidative hierarchy’ (21, 34). Jebb et al.(21) showed in a 12-d controlled overfeeding study that fuel selection in response to overfeeding is dominated by CHO intake and an increase in CHO oxidation produces a counterregulatory suppression of fat oxidation even in the presence of high fat intake. This study showed a progressive, significant, linear increase in carbohydrate oxidation which in each phase differed from carbohydrate intake by less than 0.5MJ/day (some of which can be accounted for by measurement imprecision) reiterating the precise control of carbohydrate balance. The increase in fat intake was not tracked by fat oxidation and fat was thus preferentially stored. The fat storage was primarily derived from the diet with no evidence of net *de novo* lipogenesis because respiratory quotient (RQ) values were below 1 and metabolic experiments have shown that *de novo* lipogenesis is, under most circumstances, a minor component in humans (41). The increase in energy intake produced a state of positive nitrogen balance and a subsequent increase in lean mass as a consequence of the gain in body weight.

The cost of tissue accretion per kg of body weight in our calculations gives a figure of 19.2 MJ/kg which is almost identical to the 17.6 MJ/kg obtained by Ravussin et al. The theoretical cost of weight gain calculated using the gross energy content of FM and protein in FFM (20.6%) was very close to the observed cost and they differed by only 9%. A similar difference was observed in a previous overfeeding study conducted in our laboratory (8). These calculations are necessarily somewhat crude because, although energy intake was assessed continuously energy expenditure was only measured on 7 occasions by calorimetry.
and twice by DLW necessitating an assumption of linear changes between measurements. Nonetheless these calculations of the energy cost of weight gain using provide further evidence against the existence of energy dissipative mechanisms because most of the weight change was explained by the cost of adipose and lean tissue deposition and by the increased energy expenditure associated with weight gain. The errors were close to 10% which is comfortably within the limits of precision of the various techniques employed.

**CONCLUSIONS**

This detailed experimental study has re-emphasized the very limited ability of humans to compensate for episodes of overfeeding through autoregulatory elevations in energy expenditure (23, 42). This result is teleologically predictable given that the thermogenic dissipation of even a 20% energy excess would put humans (with their small surface area to volume ratio) under considerable thermal stress. Instead the energy and substrate load is disposed of by substantial down-regulation of fat oxidation and resultant fat storage. The energy cost of weight gain was very close to the excess energy provided and thus does not support the existence of significant dissipative mechanisms to offset overeating in lean men. These results support the current conclusions from genetic studies in which all known causes of human obesity are related to defects in the regulation of appetite and the intake side of the energy balance equation (32). Finally, we have shown that the available energy is preferentially directed towards abdominal visceral fat, which has important implications for the development of the metabolic complications of weight gain.

**ACKNOWLEDGMENTS**
We are grateful to Anthony Wright for the stable isotope analysis, Elaine Collard for the preparation of the diets and Sri Aitken for assistance with the MR Body Fat Analysis. Also to the staff members, including night nurses, who supervised subjects in the calorimeter and metabolic suite.
REFERENCES


Figure Legends

**Figure 1:** Cumulative changes in body composition using a 4-C model (weight, fat mass, total body water and protein) in five subjects completing the study. Overfeeding phases= +20%, +40%, +60%; *Ad libitum* = AL. Baseline values were: Weight= 68.69±4.39 kg; Fat Mass= 14.62±3.26 kg; Protein= 11.11±0.59 kg; Total body water=39.62±1.92 kg. Data shown as mean ± s.e. *p<0.05 (relative to baseline).

**Figure 2:** Absolute values and changes (Δ) in body composition at the end of +60% overfeeding in 5 subjects. Total body fat was assessed by a 4-compartment model (4-C). FM_{BL} and FFM_{BL} are baseline values. Segmental measures of body composition were assessed by DXA (trunk, arms and legs) and MRI (subcutaneous fat mass = SCFM, visceral fat mass = VIFM). Δ% = [(60 % - baseline)/baseline]*100. L = left, R = right.

**Figure 3:** Change in energy intake (EI) relative to baseline during the study. White circles show when subjects where in the calorimeters. Each circle during the *ad libitum* phases represents a day period (some EI data during the last *ad libitum* phase are not available). * Each circle during the overfeeding phases (+20%OF, +40%, +60%) represents a week period. Values expressed as mean ± standard error.

**Figure 4:** Macronutrient oxidation and intake (MJ/day) for carbohydrate (CHO), fat (FAT) and protein (PRO) at the end of baseline and end of overfeeding phases (20%OF, 40%OF, 60%OF) measured by whole-body indirect calorimetry. One subject did not complete the study and was excluded from this analysis. * p<0.05
Table 1: Energy and macronutrient content of diets and snacks provided during the baseline and overfeeding phases.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>20 % OF</th>
<th>40 % OF</th>
<th>60 % OF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diet</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (MJ/d)</td>
<td>11.0 ± 0.4</td>
<td>13.5 ± 0.4</td>
<td>15.6 ± 0.5</td>
<td>17.8 ± 0.7</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>85 ± 4 (13 %)</td>
<td>101 ± 3 (13 %)</td>
<td>112 ± 4 (12 %)</td>
<td>126 ± 5 (12 %)</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>120 ± 4 (40 %)</td>
<td>158 ± 6 (43 %)</td>
<td>196 ± 6 (46 %)</td>
<td>231 ± 8 (48 %)</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>322 ± 16 (47 %)</td>
<td>375 ± 13 (44 %)</td>
<td>409 ± 18 (42 %)</td>
<td>446 ± 20 (40 %)</td>
</tr>
<tr>
<td>Energy Density (MJ/100g)</td>
<td>2.0</td>
<td>2.1</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td><strong>Snack</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (MJ/d)</td>
<td>-</td>
<td>2.0</td>
<td>4.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>-</td>
<td>11 (9.5 %)</td>
<td>21 (9 %)</td>
<td>33 (9.5 %)</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>-</td>
<td>32 (60 %)</td>
<td>64 (59.5 %)</td>
<td>97 (60 %)</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>-</td>
<td>38 (30.5 %)</td>
<td>78 (31.5 %)</td>
<td>114 (30.5 %)</td>
</tr>
</tbody>
</table>

The percent values in brackets are the proportion of energy provided by each macronutrient for each diet or snack. Data for the diets are shown as mean ± standard deviation. Data were based on UK food composition tables (19).
Table 2: Individual and mean changes in body weight and fat mass during the overfeeding and *ad-libitum* phases

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>AGE</th>
<th>Height</th>
<th>BMI</th>
<th>Baseline</th>
<th>+20% OF</th>
<th>Ad-Lib</th>
<th>+40% OF</th>
<th>Ad-Lib</th>
<th>+60% OF</th>
<th>Ad-Lib</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(years)</td>
<td>(m)</td>
<td>(kg/m²)</td>
<td></td>
<td>(kg)</td>
<td></td>
<td>(kg)</td>
<td></td>
<td>(kg)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>39</td>
<td>1.83</td>
<td>21.00</td>
<td>70.34 (12.77)</td>
<td>71.20 (14.59)</td>
<td>71.24</td>
<td>72.17 (15.27)</td>
<td>72.30</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>1.73</td>
<td>18.80</td>
<td>56.07 (9.09)</td>
<td>57.00 (7.82)</td>
<td>58.38</td>
<td>-</td>
<td>60.57 (10.4)</td>
<td>59.70</td>
<td>62.89 (9.82)</td>
</tr>
<tr>
<td>3</td>
<td>58</td>
<td>1.83</td>
<td>24.14</td>
<td>80.88 (27.12)</td>
<td>81.41 (25.76)</td>
<td>82.40</td>
<td>-</td>
<td>83.91 (26.58)</td>
<td>84.69</td>
<td>88.80 (30.69)</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>1.78</td>
<td>20.89</td>
<td>66.20 (13.83)</td>
<td>67.40 (16.84)</td>
<td>65.49</td>
<td>-</td>
<td>69.48 (17.96)</td>
<td>66.60</td>
<td>71.40 (18.92)</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>1.74</td>
<td>21.23</td>
<td>64.30 (9.7)</td>
<td>63.70 (10.25)</td>
<td>64.20</td>
<td>-</td>
<td>65.80 (10.28)</td>
<td>66.40</td>
<td>68.80 (13.44)</td>
</tr>
<tr>
<td>6</td>
<td>40</td>
<td>1.81</td>
<td>23.19</td>
<td>76.00 (13.38)</td>
<td>77.30 (12.88)</td>
<td>76.48</td>
<td>-</td>
<td>79.50 (14.24)</td>
<td>79.10</td>
<td>81.50 (16.82)</td>
</tr>
<tr>
<td>X ± SD</td>
<td>43.33±10.61</td>
<td>1.80±0.03</td>
<td>21.90±1.90</td>
<td>68.96±8.81 (14.31±6.57)</td>
<td>69.66±8.94 (14.69±6.28)</td>
<td>69.69±8.78 (14.78±6.05)</td>
<td>-</td>
<td>71.90±8.64 (15.78±6.05)</td>
<td>71.46±9.18 (17.93±7.92)</td>
<td>71.96±10.10 (16.84±8.37)</td>
</tr>
</tbody>
</table>

Values expressed as mean (X) ± standard deviation (s.d). Body mass index (BMI) = weight (kg)/height² (m); OF = overfeeding; Ad-Lib = *ad libitum*

*Subject 1 did not complete the study.

*p<0.05; †p<0.01; ‡p<0.001 (relative to baseline).
# Table 3: Measurement of energy expenditure using indirect calorimetry and doubly labelled water.

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>Baseline</th>
<th>+20 %</th>
<th>Ad-Lib</th>
<th>+40 %</th>
<th>Ad-Lib</th>
<th>+60 %</th>
<th>Ad-Lib</th>
<th>Baseline</th>
<th>+60 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>10.8</td>
<td>11.0</td>
<td>11.5</td>
<td>11.3</td>
<td>11.4</td>
<td>11.8</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11.4</td>
<td>11.5</td>
<td>11.7</td>
<td>11.4</td>
<td>12.7</td>
<td>11.2</td>
<td>11.0</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>10.3</td>
<td>10.4</td>
<td>10.1</td>
<td>10.8</td>
<td>9.9</td>
<td>11.4</td>
<td>9.3</td>
<td>11.4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>11.7</td>
<td>11.9</td>
<td>12.2</td>
<td>12.8</td>
<td>13.2</td>
<td>12.6</td>
<td>12.6</td>
<td>9.8</td>
<td>12.9</td>
</tr>
<tr>
<td>6</td>
<td>12.5</td>
<td>12.7</td>
<td>12.8</td>
<td>13.0</td>
<td>12.8</td>
<td>14.3</td>
<td>12.5</td>
<td>11.7</td>
<td>13.6</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>11.4±0.7</td>
<td>11.7±0.6</td>
<td>11.5±0.9</td>
<td>11.9±0.7</td>
<td>11.6±1.0</td>
<td>12.7±1.1</td>
<td>11.4±1.3</td>
<td>11.1±0.7</td>
<td>12.9±0.8</td>
</tr>
</tbody>
</table>

OF = overfeeding; Ad-Lib = ad libitum;
Values expressed as mean (X) ± standard deviation (SD).

Subject 4701 did not complete the study

*p<0.05;  *p<0.01;  *p<0.001 (relative to baseline).
Table 4: Calculation of cost of weight gain for each overfeeding phase (using calorimetry TEE) and for the whole study (using doubly labelled water TEE) in five lean healthy men

<table>
<thead>
<tr>
<th></th>
<th>OF+20%CAL</th>
<th>OF +40%CAL</th>
<th>OF +60%CAL</th>
<th>OF TotalDLW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight Gain (kg)</td>
<td>0.67</td>
<td>2.49</td>
<td>2.82</td>
<td>8.81</td>
</tr>
<tr>
<td>Fat Mass Gain (kg)</td>
<td>0.08</td>
<td>1.18</td>
<td>2.04</td>
<td>3.31</td>
</tr>
<tr>
<td>Protein Gain (kg)</td>
<td>0.12</td>
<td>0.27</td>
<td>0.16</td>
<td>0.55</td>
</tr>
<tr>
<td>Energy deposited as fat (= Δ FM x 38.9 MJ)</td>
<td>3.34</td>
<td>45.98</td>
<td>79.58</td>
<td>128.91</td>
</tr>
<tr>
<td>Energy deposited as protein (= Δ protein x 23.5 MJ)</td>
<td>2.83</td>
<td>6.34</td>
<td>3.77</td>
<td>12.95</td>
</tr>
<tr>
<td>Tissue deposition sub-total (=3+4)</td>
<td>6.18</td>
<td>52.32</td>
<td>83.36</td>
<td>141.86</td>
</tr>
<tr>
<td>Increase in calEE or TEE</td>
<td>4.35</td>
<td>9.97</td>
<td>19.72</td>
<td>69.30</td>
</tr>
<tr>
<td>Total deposited and expended (=5+6)</td>
<td>10.53</td>
<td>62.30</td>
<td>103.08</td>
<td>211.16</td>
</tr>
<tr>
<td>Total additional energy consumed</td>
<td>37.51</td>
<td>82.69</td>
<td>118.69</td>
<td>238.90</td>
</tr>
<tr>
<td>Unexplained (=8-7)</td>
<td>26.98</td>
<td>20.39</td>
<td>15.60</td>
<td>27.74</td>
</tr>
<tr>
<td>Unexplained (%)</td>
<td>71.92</td>
<td>24.66</td>
<td>13.14</td>
<td>11.61</td>
</tr>
<tr>
<td>Energy equivalent of weight gain (MJ/kg)</td>
<td>38.55</td>
<td>29.20</td>
<td>35.09</td>
<td>19.25</td>
</tr>
</tbody>
</table>

Δ FM = gain in fat mass; TEE= total energy expenditure; CAL = calorimetry; DLW= doubly labelled water.

Increase in calEE or TEE was calculated as the difference between the average calEE or TEE and the baseline calEE or TEE multiplied by number of days \([(EE_{OF+EE_{Baseline}})/2]-EE_{baseline}] *Number of days

The synthesis costs of fat and protein have already been accounted for in the measurement of TEE

**Energy equivalent of weight gain (MJ/kg) = Total additional energy consumed/ Increase in calEE or TEE**

CalEE measured using whole body indirect calorimetry was used to calculate the cost of weight gain for each overfeeding phase and the changes in body stores and energy expenditure were computed as follows:

- **OF+20%CAL = OF+20% - Baseline**
- **OF+40%CAL = OF+40% - OF+20%** (includes changes occurring during ad libitum phase 1)
- **OF+60%CAL = OF+60% - OF+40%** (includes changes occurring during ad libitum phase 2)

TEE measured using doubly labelled water (DLW) was used to calculate the cost of weight gain for the whole study and the changes in body stores and energy expenditure were computed as follows:

- **OFTotalDLW = OF+60% - Baseline**
**Trunk**

- FM_left (kg) = 4.5
- FM_right (kg) = 27.7
- Δ FM (kg) = +2.7
- Δ FM (%) = +60.3
- Δ FFM (kg) = +1.2

**Arm (L + R)**

- FM_left (kg) = 1.8
- FM_right (kg) = 5.7
- Δ FM (kg) = +0.5
- Δ FM (%) = +27.9
- Δ FFM (kg) = +0.6

**MRI Scan**

- Δ SC FM (cm²) = +47.7
- Δ SC FM (%) = +13.3
- Δ VFM (cm²) = +38.5
- Δ VFM (%) = +32.6

**Legs (L + R)**

- FM_left (kg) = 4.5
- FM_right (kg) = 18.0
- Δ FM (kg) = +0.7
- Δ FM (%) = +16.8
- Δ FFM (kg) = +0.4