Insulin resistance in obesity: body-weight or energy balance?

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Abstract

Weight reduction is recommended for the treatment of subjects with insulin resistance (IR) syndrome; however, the relative importance of the decrease in body fat or the negative energy balance achieved during a hypo-energetic diet in the improvement of this metabolic syndrome is still debated. Therefore, we undertook to study their relative impact on amelioration of the metabolic abnormalities associated with IR in obese subjects.

Twelve obese subjects (six males and six females, mean $\pm$ s.d. body mass index 36.1 ± 4.7 kg/m²) aged 38–57 years were investigated. During the first phase they were fed a hypo-energetic diet for 6 weeks (week 0–6). During the second phase, lasting 4 weeks (week 6–10) they consumed an iso-energetic diet. During the third phase (week 10–16) the subjects were put again on a hypo-energetic diet. Insulin sensitivity (SI) was assessed by an insulin-enhanced, frequently sampled i.v. glucose tolerance test with minimal model analysis. All subjects

reduced weight during both hypo-energetic periods: 5.49 ± 0.75 and $2.32 \pm 0.37\%$, means $\pm$ s.e.m., $P < 0.005$, week 0–6 and 10–16 respectively. One-third of this loss was achieved within the first week of each period. SI increased by 353 ± 121 and $147 \pm 38\%$ ($P < 0.005$), means $\pm$ s.e.m., at the end of both hypo-energetic periods (week 6 vs 0 and 16 vs 10 respectively). Two-thirds of this improvement were observed within the first week of each period (week 1 vs 0 and 11 vs 10 respectively). During the iso-energetic weight-maintaining period (week 10 vs 6), SI decreased by $43.5 \pm 7.9\%$ ($P < 0.002$). Serum levels of leptin and triglyceride followed a similar pattern, but to a lesser extent.

It may be concluded that negative energy balance is more effective when compared with maintaining a stable lower weight in achieving an improvement in the metabolic parameters of the IR syndrome.

Journal of Endocrinology (2001) **171**, 293–298

Introduction

Obesity threatens to become the 21st century's leading health problem (Rosenbaum *et al.* 1997). Several metabolic abnormalities are associated with obesity including: atherogenic dyslipidemia, elevated blood pressure, hypercoagulable state and insulin resistance (IR) (Bray 1996, Smith 1996). Most investigators believe that IR and the associated hyperinsulinemia are the cause of this metabolic syndrome, which is common in persons who develop premature coronary heart disease (Reaven 1988, Despres *et al.* 1996). IR is a heterogeneous syndrome with both genetic (Haffner *et al.* 1989, Dunaif 1997) and environmental (Moller & Flier 1991, Facchini *et al.* 1992, Assali *et al.* 1999) factors playing key roles in its development. Although several reports have indicated that patients with IR are obese (particularly visceral obesity), it has been difficult to determine whether or not obesity is the primary abnormality in the pathogenesis of the syndrome

(Kannel *et al.* 1979, Henry *et al.* 1986, Hale *et al.* 1988, Reaven 1988, Franssila-Kallunki *et al.* 1992). Nagulesparan *et al.* (1980) demonstrated a positive correlation between IR and body weight; however, the amount of body fat is clearly not the sole determinant of IR. Mingrone *et al.* (1997) and unpublished data cited by Schwartz & Seeley (1997) suggest that the improvement in IR is not related to the weight loss itself but to other changes that occur during this process. Weinstock *et al.* (1998) reported that the insulin levels in obese women who maintained 10% loss of their body weight returned to pretreatment levels. Furthermore, obese and lean subjects have a similar neuroendocrine response to active weight loss, and in both resumption of food intake reverses most of this effect, even if the lost weight is not regained (Schwartz & Seeley 1997).

Since the centrally acting negative-feedback signals, leptin and insulin, are sensitive to both body fat mass and energy balance, we elected to study these and other

metabolic variables associated with IR in obese subjects during two states of energy balance: hypo-energetic (active weight loss) and iso-energetic (at stable lower weight). Elucidation of their relative capacity to ameliorate the metabolic abnormalities associated with IR in obese subjects carries important therapeutic implications.

Methods

Subjects

Twelve healthy obese subjects, six men and six women, aged 38–57 years, with a body mass index (BMI) of 36.1 ± 4.7 kg/m² were recruited by advertisement on the hospital bulletin board (Table 1). Because of the known effects of the menstrual cycle on plasma lipids and insulin sensitivity (SI) (Jones *et al.* 1988, Valdes & Elkind-Hirsch 1991), only menopausal women were included. None had a history of cancer, heart, liver, kidney, endocrine, blood, lung, gastrointestinal and neurologic disease, nor detected abnormalities on physical examination and routine laboratory testing. All subjects were normotensive (blood pressure less than 140/90 mmHg), took no regular medications nor used medications known to affect weight or energy expenditure (e.g. hormonal replacement therapy, thyroid hormones or anorexigenic agents). The study had been approved by the hospital Ethics Committee and all subjects signed an informed consent.

Study protocol

The subjects were asked to maintain their usual physical activity, food intake, smoking habits and daily routine. After the initial workup and 4 weeks of weight stabilization, the subjects were randomly prescribed one of two hypo-energetic diets: low calorie diets (LCD) or very low calorie diets (VLCD) (one-third and two-thirds deficit of habitual energy intake respectively), consisting of 3350–11 300 kJ/day (50–55% carbohydrate, 30% fat, 15–20% protein and <300 mg cholesterol/day), divided into four meals, for 6 weeks (first phase: week 0–6). The second phase consisted of 4 weeks (week 6–10) of a weight-maintaining iso-energetic diet. During the third phase (week 10–16) the subjects consumed the alternate hypo-energetic diet to achieve a second phase of active weight loss (those who were started on LCD were switched to VLCD and vice versa). Compliance with the study protocol and dietary habits were confirmed by weekly interviews and dietary recalls. All subjects maintained their daily routine and did not change their smoking habits during the entire study period. None of the subjects was taking part in physical fitness programs.

The subjects were studied at baseline (week 0); after 1 week (end of week 1) and at the end of the first period (end of week 6) of the hypo-energetic diet (LCD or

VLCD); after 4 weeks of the iso-energetic diet (end of week 10); after 1 week (end of week 11) and at the end (week 16) of the second (alternate VLCD or LCD) hypo-energetic diet (end of week 16). At each of these visits, conducted at 0800 h, after 12 h overnight fast, the subjects were weighed, physically examined, blood withdrawn and the i.v. glucose tolerance test performed.

Measurement and analysis

Plasma cholesterol, triglyceride, and high-density lipoprotein (HDL) levels were determined by enzymatic methods (Boehringer, Mannheim, Germany). Plasma lipoproteins were determined by β -quantification. The SI-index was assessed by an insulin-enhanced, frequently sampled i.v. glucose tolerance test with minimal model analysis (Welch *et al.* 1990). In brief, after a 12 h overnight fast and 30 min recumbence, i.v. lines were inserted into the antecubital veins, one for blood sampling and one for glucose and insulin administration. Glucose (0.3 g/kg) and insulin (0.03 U/kg) were injected i.v. at 0 and 20 min respectively, and blood samples for glucose and insulin determination were collected at –5, –1, 2, 4, 6, 8, 10, 12, 14, 16, 19, 22, 23, 25, 27, 30, 40, 50, 90, 120 and 180 min. Blood glucose was determined by glucose-oxidase (Boehringer), serum insulin by RIA (Sorin, Biomedica, Sallugia, Italy) and leptin by RIA (Linco Research Inc., St Charles, MO, USA).

Data analysis

Data are presented as means $\pm$ S.D. or means $\pm$ S.E.M. Because of the large variability in the baseline values for anthropometric and metabolic parameters (Table 1), we elected to compute for each individual the percent differences from baseline values (Δ) and calculate their means and trends. As we observed no statistically significant differences between the effects of the two hypo-energetic diets (LCD and VLCD) on the studied metabolic parameters during the entire study ($P > 0.05$), and in order to simplify the presentation, we elected to pool the data. We estimated the significance of the observed differences between men and women, and within periods, by Student's *t*-test for unpaired and paired data respectively. We used repeated measures ANOVA to compare different periods and alternate diets, and Pearson correlation coefficient to study the degree of association between changes in SI and weight. $P \leq 0.05$ was considered significant.

Results

The baseline characteristics of the studied subjects are detailed in Table 1. The women recruited for this study were 10 years older than the men, to avoid the putative

Table 1 Baseline characteristics of the studied subjects (means $\pm$ S.D. (range))

	Males (n=6)	Females (n=6)	Total (n=12)
Age (years)	44.6 $\pm$ 5.2 (38–46)	54.0 $\pm$ 2.8 (50–57)	49.7 $\pm$ 6.2*
Body weight (kg)	109.3 $\pm$ 22.4 (91–149)	95.1 $\pm$ 10.5 (81–107)	102.2 $\pm$ 18.3
Height (cm)	175.6 $\pm$ 8.8 (163–190)	160.7 $\pm$ 3.1 (158–165)	168.1 $\pm$ 10.0*
BMI (kg/m ²)	35.2 $\pm$ 4.6 (31.5–41.3)	36.9 $\pm$ 5.1 (29.9–43.0)	36.1 $\pm$ 4.7
Fasting glucose (mmol/l)	5.33 $\pm$ 0.41 (4.89–5.94)	5.55 $\pm$ 0.66 (4.89–6.77)	5.45 $\pm$ 0.54
Leptin (ng/ml)	13.9 $\pm$ 6.6 (6.5–21.2)	31.3 $\pm$ 8.3 (19.8–42.4)	22.6 $\pm$ 11.6*
Fasting insulin (pmol/l)	118 $\pm$ 74 (65–260)	111 $\pm$ 52 (57–197)	114 $\pm$ 61
Insulin sensitivity index (10 ⁻⁴ $\times$ min ⁻¹ /μU per ml)	1.04 $\pm$ 1.15 (0.05–2.3)	1.02 $\pm$ 1.80 (0.05–4.4)	1.03 $\pm$ 1.44
Cholesterol (mmol/l)			
Total	5.95 $\pm$ 1.04 (4.89–7.16)	6.32 $\pm$ 0.93 (4.94–7.68)	6.14 $\pm$ 0.96
VLDL	0.83 $\pm$ 0.26 (0.59–1.27)	1.00 $\pm$ 0.45 (0.44–1.66)	0.91 $\pm$ 0.36
LDL	3.61 $\pm$ 1.06 (2.22–4.78)	3.95 $\pm$ 0.77 (2.97–5.09)	3.78 $\pm$ 0.90
HDL	1.17 $\pm$ 0.13 (0.96–1.27)	1.28 $\pm$ 0.34 (0.93–1.89)	1.22 $\pm$ 0.25
Triglyceride (mmol/l)	2.56 $\pm$ 0.62 (2.07–3.73)	2.38 $\pm$ 0.96 (1.59–3.61)	2.47 $\pm$ 0.75

* $P < 0.05$, difference between males and females by unpaired Student's *t*-test.

effect of the menstrual cycle on studied metabolic parameters. Their average weight and height were lower, but their BMI was similar to those of the men. The baseline metabolic values besides those of leptin (which was more than 2-fold in women) were similar in both genders.

Body weight

All subjects lost weight during the study, with an average of 8.7 kg ($P < 0.05$). Men and women showed similar rates during all periods ($P > 0.5$ (not shown)). Although the subjects starting on a VLCD regimen showed a tendency for a higher rate in weight reduction when compared with the subjects starting on an LCD, 2.19 vs 1.43% within the first week (week 0–1), 6.58 vs 4.55% during the first hypo-energetic period (week 0–6) and 9.40 vs 8.53% during the whole study (week 0–16) (percent of their initial body weight respectively), these differences did not reach statistical significance ($P > 0.5$).

The body weight of the entire group was reduced by an average of 5.49 $\pm$ 0.75%, means $\pm$ S.E.M., during the first hypo-energetic period (week 0–6, from 102.2 $\pm$ 5.3 to 96.5 $\pm$ 5.1 kg, $P = 0.003$) and by 2.32 $\pm$ 0.37% during the second hypo-energetic period (week 10–16, from

95.7 $\pm$ 5.2 to 93.5 $\pm$ 5.1 kg, $P = 0.001$). One-third of this weight reduction was observed during the first week of these periods (week 1 and 11) (Table 2 and Fig. 1). Although the subjects were encouraged to consume an iso-energetic diet during the interim iso-energetic period (week 6–10), most continued to lose weight, although at a reduced rate, with an average of 0.91 $\pm$ 0.42% (96.5 $\pm$ 5.1 to 95.7 $\pm$ 5.2 kg, $P = 0.037$) during these 4 weeks (Table 2).

SI

Fasting serum glucose and insulin did not change significantly during the entire study (Table 2). The SI-index increased by 475 $\pm$ 196% during the 16 weeks of study, from 1.03 $\pm$ 0.43 to 4.78 $\pm$ 1.06 (10⁻⁴ $\times$ min⁻¹/μU per ml), means $\pm$ S.E.M. ($P < 0.005$). The increase in SI-index was 353 $\pm$ 121% at the end of the first hypo-energetic period (week 0–6, from 1.03 $\pm$ 0.43 to 3.57 $\pm$ 0.58 (10⁻⁴ $\times$ min⁻¹/μU per ml), $P = 0.0017$), and by 147 $\pm$ 38% ($P = 0.002$) by the end of the second hypo-energetic period (week 10–16, from 1.96 $\pm$ 0.50 to 4.78 $\pm$ 1.06 (10⁻⁴ $\times$ min⁻¹/μU per ml), $P = 0.0016$). Two-thirds of this increase in the SI-index were observed during the first week of each hypo-energetic period (week 1 and 11)

Table 2 Changes in studied parameters during the study expressed as $\Delta\%$ during each period ($n=12$). The first hypo-energetic dietary period (VLCD or LCD) lasted for 6 weeks (0–6), followed by 4 weeks (6–10) of iso-energetic intake and a second period of hypo-energetic dietary intake (LCD or VLCD) (weeks 10–16). Blood samples and i.v. glucose tolerance tests were obtained at the designated times. Values for each individual were calculated as $\Delta\%$ (i.e. for period 0–1 ((value for week 1 – value for week 0)/value for period 0). These values were pooled for the whole group and expressed as means $\pm$ S.E.M.

	Hypo-energetic		Iso-energetic	Hypo-energetic	
	0–1 weeks	0–6 weeks	6–10 weeks	10–11 weeks	10–16 weeks
Body weight	$-1.81 \pm 0.24^{**}$	$-5.49 \pm 0.75^{**}$	$-0.91 \pm 0.42^{**}$	$-0.75 \pm 0.20^*$	$-2.32 \pm 0.37^{**}$
Glucose	-2.2 ± 2.8	-0.03 ± 2.7	-2.9 ± 3.2	$+1.1 \pm 3.1$	$+0.9 \pm 4.0$
Insulin	$+9.3 \pm 12.4$	0.4 ± 13.7	$+4.2 \pm 12.1$	7.1 ± 8.4	$+3.2 \pm 7.7$
Leptin	$-22.0 \pm 3.2^{**}$	$-37.7 \pm 5.1^{**}$	$+18.6 \pm 14.9$	-3.3 ± 8.6	$+4.5 \pm 16.5$
SI-index	$+219 \pm 63^{**}$	$+353 \pm 121^{**}$	$-44 \pm 8^{**}$	$+103 \pm 23^{**}$	$+147 \pm 38^{**}$
Triglyceride	$-19.5 \pm 6.4^{**}$	$-22.3 \pm 7.6^*$	$+10.5 \pm 7.8$	$+0.8 \pm 8.4$	-3.9 ± 10.8
Cholesterol	-3.1 ± 3.4	-5.1 ± 4.3	$+5.0 \pm 5.0$	$+0.3 \pm 3.8$	-4.5 ± 3.6
VLDL	$-19.3 \pm 6.4^{**}$	$-22.5 \pm 7.6^*$	-10.7 ± 7.9	$+2.3 \pm 8.6$	$+2.3 \pm 11.2$
LDL	$+5.5 \pm 6.2$	$+3.5 \pm 8.1$	$+4.5 \pm 6.2$	$+0.6 \pm 4.8$	-6.6 ± 4.0
HDL	$-9.3 \pm 3.5^*$	-5.2 ± 4.5	$+4.7 \pm 3.7$	-0.4 ± 2.1	-0.6 ± 2.4

The statistics reflects comparison of the absolute values within each designated period by paired two-tailed Student's test: $^*P<0.05$, $^{**}P<0.005$.

Figure 1 Changes of weight, SI (units $10^{-4} \times \text{min}^{-1}/\mu\text{U}$ per ml), triglyceride and leptin during study periods. Values were pooled for the whole group and expressed as means $\pm$ S.E.M., $n=12$. The statistics reflect comparison of the absolute values periods: 0–1, 0–6, 6–10, 10–11 and 10–16 weeks by a paired two-tailed Student's *t*-test. $^*P<0.05$, $^{**}P<0.005$.

(Table 2 and Fig. 1), while the body weight decreased by less than 2 kg (Fig. 1). During the iso-energetic period (week 6–10), although none of the subjects gained weight, we observed a decrease in the SI-index (from 3.57 ± 0.57 to 1.96 ± 0.50 ($10^{-4} \times \text{min}^{-1}/\mu\text{U}$ per ml), week 6 and 10 respectively, $P=0.0011$) (Table 2, Fig. 1).

Leptin

Serum leptin levels decreased in both men and women during the study period by 33.14 ± 8.04 and $34.24 \pm 5.41\%$, means $\pm$ S.E.M. respectively (from 13.87 ± 1.99 to

9.43 ± 1.70 and from 31.28 ± 2.49 to 20.88 ± 2.83 ng/ml respectively, $P=0.0014$, paired Student's *t*-test). The major part of this decrease occurred during the first hypo-energetic diet (0–6 weeks, from 13.87 ± 1.99 to 7.53 ± 0.93 and 31.28 ± 2.49 to 21.40 ± 3.29 ng/ml in men and women respectively, $P=0.0007$ (42.3 ± 3.9 and $33.0 \pm 5.8\%$ respectively). Fifty-eight percent of this decrease were observed during the first week (week 1) (Table 2 and Fig. 1). During the iso-energetic period (week 6–10), the leptin levels increased by 18% ($P=0.3$), and tended to decrease during the second hypo-energetic period ($P=0.4$).

Plasma lipids and lipoproteins

The changes observed in plasma total and low-density lipoprotein (LDL) cholesterol showed no consistent pattern and did not reach statistical significance (Table 2). Plasma HDL cholesterol showed a trend for decrease, reaching its lowest level within the first week of dieting (week 0–1), when it dropped from 1.22 ± 0.08 to 1.10 ± 0.06 mmol/l ($P=0.021$), showing a tendency for increase thereafter (Table 2). Plasma triglyceride and very-low-density lipoprotein (VLDL) cholesterol decreased within the first week of the hypo-energetic diet (week 0–1) by $19.5 \pm 6.4\%$ ($P=0.003$) and $19.3 \pm 6.4\%$ ($P=0.003$) respectively. They continued to decrease during the next 5 weeks but increased thereafter (Table 2 and Fig. 1).

Discussion

The major observation of the present study indicates that energy restriction alone (i.e. negative energy balance) induces marked improvement in IR, serum leptin and triglyceride levels long before obese subjects lose a substantial amount of weight. Removal of the energy restriction even for a few weeks, while consuming an iso-energetic diet, leads to reversal of most of these beneficial effects, although the body weight did not increase. Beneficial effects of weight loss on glucose metabolism, plasma lipids and coronary artery disease risk in obese subjects have been well documented (Moller & Flier 1991, Bray 1996, Mingrone *et al.* 1997, Rosenbaum *et al.* 1997). Such improvements, as shown also in our study, have been observed whether weight was lost with an LCD (4185–6280 kJ/day) or a VLCD (1675–3350 kJ/day) diet (Wing *et al.* 1991, 1994).

Previous studies have focused on the role of loss in body fat during weight reduction but not on the role of energy balance. The glucose, insulin and lipoprotein abnormalities associated with obesity are attributed, in part, to overweight (Smith 1996, Rosenbaum *et al.* 1997); however, the results of the present study indicate that the decrease in both IR and serum leptin that accompany weight loss are related to the energy deficiency associated with active weight loss, and that this improvement is partially abolished during restoration of energy balance and stabilization at a lower weight.

Most of the observed improvement in SI is achieved during the first week of energy restriction, well before considerable weight is lost. During the first 6 weeks of study the subjects lost a mean of 5.49% of their body weight and their SI improved by a mean of 353%. Almost two-thirds of the improvement in SI had been achieved during the first week, when the mean weight loss was just 1–2 kg (one-third of the weight lost during the entire period). Similar findings were observed during the second phase of energy deficiency. These findings are consistent

with previous studies (Wing *et al.* 1991, 1994, Weinstock *et al.* 1998) and indicate that the observed improvement in SI during weight loss is regulated by factors other than the adipose tissue mass.

It is highly unlikely that small changes in body weight could have been responsible for the profound decrease in IR, since caloric restriction alone is known to induce improvements in insulin and glucose levels, long before subjects have lost significant amounts of weight. Unlike previous studies, we observed that when the energy restriction is terminated, resumption of weight-maintaining food intake reverses most of the improvement in SI, even if the lost weight is not regained.

The occurrence of IR or its severity in obesity appears to be acquired to a large extent, since non-obese individuals develop IR upon weight gain (Campbell & Gerich 1990, Caro 1991, Eckel 1992). Insulin-mediated glucose disposal drops by 30–40% when an individual is 40% over his ideal body weight (Golay *et al.* 1988, Pories *et al.* 1995). Previous studies have shown that weight reduction leads to permanent improvement in glucose tolerance and increased peripheral sensitivity to insulin action in obese subjects (Henry *et al.* 1986, Hale *et al.* 1988, Franssila-Kallunki *et al.* 1992) and can restore fertility in obese women with polycystic ovary syndrome (Kiddy *et al.* 1992). Recently (Schwartz & Seeley 1997) it was suggested that the amount of body fat is not the sole determinant of the neuroendocrine response to weight loss. This response, which includes lowering of insulin and leptin, is sensitive to both fat stores and energy balance. Hence it was suggested that active weight loss (i.e. negative energy balance) lowers the concentration of these hormones to values well below those observed during the weight-maintenance phase after weight reduction has been achieved when the adipose mass did not increase.

What could have been responsible for the rapid decrease in IR, observed promptly after initiating the negative energy balance? A definitive answer to this question is presently not available. It has been shown that excessive release of free fatty acids and glycerol from adipocytes into the circulation in the obese state is one of the major factors responsible for IR (Ferrannini *et al.* 1983, Svedberg *et al.* 1991). The increase in supply of free fatty acids from an enlarged fat mass leads to both an increase in oxidation of lipids, and through activation of the glucose-free fatty acid cycle (Randle *et al.* 1963, Bonadonna & Bonora 1997, McGarry & Dobbins 1999), to a decrease in the oxidation of glucose. Reversal of this cycle improves SI (Randle *et al.* 1963, Franssila-Kallunki *et al.* 1992). It is conceivable that the rapid and substantial reduction in IR within the first week of a hypo-energetic diet as observed in our study, is the result of the normalization of substrate oxidation, rather than a reduction in body fat mass.

Our study has several limitations. First we studied a small number of patients, but the prospective design resulted in consistent and significant results. The second is

that we failed to maintain a stable weight during the second phase (iso-energetic) of the study in all subjects. Although it was difficult for the subjects to avoid further weight reduction, we have observed reversal in the trend of amelioration in IR, leptin and triglyceride levels.

It may thus be concluded that negative energy balance, induced by a hypo-energetic diet, has beneficial effects in the IR syndrome before a significant reduction in body weight. Corroboration of these finding in larger groups of patients will improve our understanding of the mechanism and treatment of this devastating metabolic syndrome.

Acknowledgements

The study is part of the requirement for an MSc degree for A G, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

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Received 16 March 2001

Accepted 24 July 2001