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## Less fat reduction per unit weight loss in type 2 diabetic compared with nondiabetic obese individuals completing a very-low-calorie diet program

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### ABSTRACT

The objective was to compare weight loss and change in body composition in obese subjects with and without type 2 diabetes mellitus during a very-low-calorie diet (VLCD) program. Seventy weight-matched subjects with diabetes or normal fasting glucose (controls) participated in a 24-week VLCD study. Primary end points were changes in anthropometry, body composition, and fasting plasma insulin and  $\beta$ -hydroxybutyrate concentrations. Fifty-one subjects (24 with diabetes) completed the study. No difference in weight loss between the 2 groups at 24 weeks was found by intention-to-treat analysis. Both groups completing the study per protocol had near-identical weight change during the program, with similar weight loss at 24 weeks (diabetes:  $8.5 \pm 1.3$  kg vs control:  $9.4 \pm 1.2$  kg,  $P = .64$ ). Change in fat mass index correlated with change in body mass index (BMI) in both groups (diabetes:  $r = 0.878$ , control:  $r = 0.920$ , both  $P < .001$ ); but change in fat mass index per unit change in BMI was less in the diabetic group compared with controls ( $0.574$  vs  $0.905$  decrease,  $P = .003$ ), which persisted after adjusting for age, sex, and baseline BMI ( $P = .008$ ). Insulin concentrations remained higher and peak  $\beta$ -hydroxybutyrate concentrations were lower in the diabetic compared with the control group. While following a 24-week VLCD program, obese subjects with and without diabetes achieved comparable weight loss; but the decrease in adiposity per unit weight loss was attenuated in diabetic subjects. Hyperinsulinemia may have inhibited lipolysis in the diabetic group; however, further investigation into other factors is needed.

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## 1. Introduction

Weight loss is advocated as a cornerstone of management for all overweight and obese individuals with type 2 diabetes mellitus (T2DM) [1,2]. However, comparative studies of non-surgical and surgical weight loss interventions have suggested that overweight and obese individuals with diabetes lose less weight than those without diabetes [3–7]. One early study of behavioral and dietary intervention over 20 weeks in 12 diabetic subjects and their nondiabetic spouses found 5.9 kg less weight loss in the diabetic group [3]. Contrastingly, another study by the same group suggested that diabetic individuals can lose similar weight to nondiabetic individuals during a 16-week intervention [8].

Very-low-calorie diets (VLCDs)—defined as diets limiting energy intake to 800 kcal (3.35 MJ) per day while providing at least 50 g of high-quality protein and amino acids; essential fatty acids; and daily requirements of trace elements, vitamins, and minerals—have been advanced as a therapeutic intervention for weight loss in overweight and obese individuals with T2DM [9–11]. Previous studies comparing the efficacy of VLCDs in obese subjects with and without diabetes have been inadequate, however. An early VLCD study found less weight loss in 10 T2DM subjects compared with 5 nondiabetic subjects, but the diabetic group was less obese at baseline [12]. Another VLCD study of 7 subjects with diabetes receiving insulin therapy vs 11 non-insulin-treated diabetic and 12 control subjects suggested that insulin therapy impairs weight loss [13]. Limitations of this study included its size and more women than men in the insulin-treated group, because women may be less successful using VLCDs than men [14,15].

Past VLCD studies in obese subjects have suggested that reduction in fat mass comprises at least 75% of weight lost during these diets [16–19], although evidence for equivalent changes in body composition in diabetic vs nondiabetic obese subjects undergoing VLCDs has not been established in comparative studies.

We hypothesized that obese individuals with T2DM lose less weight than nondiabetic obese individuals following a VLCD program. As insulin inhibits lipolysis [20], high circulating insulin concentrations in diabetic subjects may attenuate the reduction in adipose tissue associated with VLCD-induced weight loss. Furthermore, hyperinsulinemia in diabetic subjects may reduce the tolerability and efficacy of VLCDs, as insulin inhibits the ketosis considered imperative in suppressing hunger during calorie restriction [21,22]. The aims of this study, therefore, were to investigate the efficacy of a VLCD program in reducing weight and adiposity in obese subjects with T2DM or normal fasting glucose over a 24-week intervention.

## 2. Methods

### 2.1. Participants

The study was a 24-week, single-center, prospective, non-randomized case-control trial. Seventy weight-matched par-

ticipants, aged between 25 and 75 years, were recruited according to body mass index (BMI) criteria for obesity, that is,  $\text{BMI} \geq 30 \text{ kg/m}^2$ . Participants were recruited between June 2007 and September 2009 from the outpatient clinics in the Endocrine Centre at Austin Health, Melbourne, Australia, from a patient database at the University of Melbourne's Department of Medicine (Austin Health) via intrahospital advertisements and word of mouth. Participants were assigned to 2 groups according to presence of T2DM and a control group with normal fasting glucose concentrations ( $<5.6 \text{ mmol/L}$ ). Within the diabetic group, 2 subgroups were later identified (post hoc): those on sulfonylureas or insulin with or without metformin (SUI) and those on diet and metformin alone (DMF). Written informed consent was obtained before commencement of the study. The study was approved by the Austin Health Human Research Ethics Committee and registered with the Australian New Zealand Clinical Trials Registry (ACTRN12607000133437).

Exclusion criteria were BMI greater than  $50 \text{ kg/m}^2$ ; current use of weight-altering medications including thiazolidinediones; type 1 diabetes mellitus; impaired fasting glucose or impaired glucose tolerance; new-onset diabetes ( $<6$  months' duration); recent smoking cessation or a plan to quit within 6 months; presence of a significant comorbidity such as stage 4 to 5 chronic kidney disease, New York Heart Association class III to IV cardiac failure, concomitant malignancy, or a significant endocrinopathy; previous failure to lose weight on VLCDs; and a history of bariatric surgery.

### 2.2. Study design

At the conclusion of baseline assessments, all participants commenced a VLCD program consisting of a 12-week intensive phase of 3 sachets or bars of Optifast (Nestlé Nutrition, Frankfurt, Germany) daily combined with a serving of salad or vegetables once daily (approximately  $800 \text{ kcal d}^{-1}/3.35 \text{ MJ d}^{-1}$ ). Participants purchased Optifast meal replacements at cost price (approximately AU \$15/wk). From week 12, participants were changed over an 8-week transition phase, under dietician supervision, to a calorie-restricted diet based on the Australian Commonwealth Scientific and Industrial Research Organization Total Wellbeing diet (approximately  $1350 \text{ kcal d}^{-1}/5.60 \text{ MJ d}^{-1}$ ) [23]. This diet was continued for 4 weeks until the end of the study. Upon commencement of the VLCD, subjects on insulin therapy had their dose approximately halved, with further adjustments made during the study to avoid hypoglycemia. No subjects ceased insulin or sulfonylurea therapy during the study. Identical information sheets and advice were given, with participants encouraged to perform 150 minutes of low- to moderate-intensity exercise per week. Clinical assessments occurred at baseline and weeks 2, 4, 8, 12, 16, 20, and 24, reflecting standard patient care in the Weight Control clinic at Austin Health.

### 2.3. Clinical, anthropometric, and laboratory assessment

All participants provided a fasting blood sample for glycated hemoglobin ( $\text{HbA}_{1c}$ ) and plasma glucose concentrations at baseline. At each visit, participants underwent a physical examination; and anthropometric data were recorded. Height

was measured to the nearest centimeter using a wall-mounted stadiometer. Weight was measured to the nearest 0.1 kg in light clothing without shoes using digital platform scales (Model 8000, Ranger Instruments, Queensland, Australia). Hip circumference and waist circumference at the level of the umbilicus were recorded to the nearest 0.5 cm with subjects standing in full expiration.

Fasting plasma glucose, insulin, C-peptide, and  $\beta$ -hydroxybutyrate concentrations were determined at each time point. Glycated hemoglobin was measured at baseline, week 12, and week 24. At baseline and the end of the study, a 24-hour urine collection was performed to quantify creatinine excretion. This allowed estimation of total body protein using the following formula:  $-10.901 + [0.0002658 \times \text{urinary creatinine (micromoles per day)}] + [0.08125 \times \text{height (centimeters)}] + [0.06141 \times \text{weight (kilograms)}]$ . This formula has been found to correlate ( $R^2 = 0.742$ ,  $P < .001$ ; Strauss, unpublished data, 2011) with total body protein determined by in vivo neutron activation analysis in our laboratory [24–26]. Urinary glucose excretion was quantified to determine daily calorie loss.

Participant satiety and hunger were quantified using a validated 100-mm visual analogue scale [27] performed in the fasting state at each clinic visit. Six questions addressed hunger or satiety over the preceding 5 minutes. Hunger scores and (100 – satiety) scores, both in millimeters, were tallied, with higher total indicating more hunger and less satiety. Subjects were asked to report time spent in daily physical activity to the nearest 30 minutes. Hypoglycemic episodes were recorded in blood glucose diaries by the diabetic group and were collated at each clinic visit. *Severe hypoglycemia* was defined as a hypoglycemic event requiring third-party assistance.

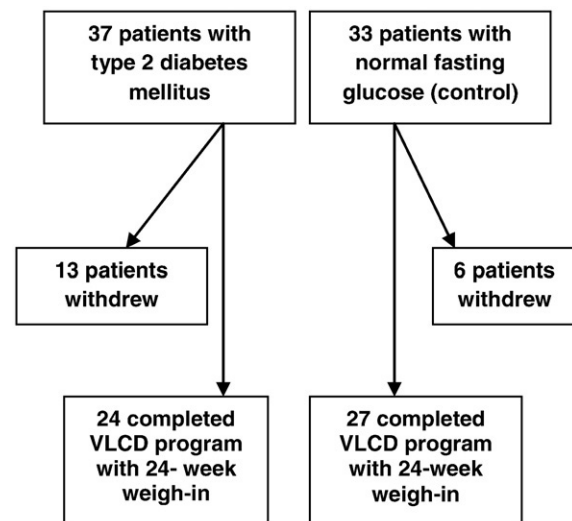
#### 2.4. Body composition assessment

Body composition was assessed at baseline and the end of study. Resting metabolic rate was estimated using a BioScan 916S bioimpedance analyzer (Maltron, Rayleigh, Essex, UK). Total body composition was assessed on the same day by dual-energy x-ray absorptiometry (DXA) scanning (DPX-L, version 1.3z; Lunar, Madison, WI). Regional fat measurements were obtained at the abdominal (android) and hip (gynoid) level using standard windows for regions of interest [28]. Appendicular lean tissue mass was calculated as the sum of upper limb lean tissue mass and lower limb lean tissue mass and used as a surrogate for total body skeletal muscle mass [29]. Corrections for height were made—by dividing by height<sup>2</sup>—for both fat mass (fat mass index, FMI) and appendicular lean tissue mass (lean tissue mass index, LTMI).

#### 2.5. Statistical analysis

Clinic-derived preliminary data supported the expectation that more than 80% of participants would lose at least 5 kg during a 24-week period. Power calculations suggested that a sample size of 24 participants in each group would be required to detect a 5-kg absolute difference (95% confidence interval) in weight loss with 80% power. Recruitment of 70 participants took into account a projected 30% dropout rate. Analysis of weight changes consisted of (a) an intention-to-treat analysis

(ITT,  $n = 70$ ), where final weights for dropouts were replaced with baseline values, and (b) a per-protocol analysis for those who completed the program (PP,  $n = 51$ , Fig. 1), where within-study missing values were replaced using linear interpolation. Continuous data (including changes between 2 time points) were analyzed using Student  $t$  test, and comparisons between independent groups were carried out using the Welch  $t$  test or analysis of variance. Except where noted, results are reported as mean  $\pm$  standard error. Categorical variables were analyzed using the  $\chi^2$  test or Fisher exact test. Repeated-measures continuous data were analyzed using linear mixed effects (LME) models estimated using restricted maximum likelihood estimators, with time and group as fixed effects (allowing for interaction between the two) and covariate weight (except for analysis of weight) and subject identifier as random effects. The LME analyses were carried out on raw data for the completers (because the analysis allows for within-study missing values). In addition, for weight, the LME analysis was also varied out on the return to baseline data. Overall significance of fixed effects was tested using Wald tests. In the plots of mean weight over time, to ensure that means were comparable (ie, to account for some missing subjects at various time points), within-study missing values were tested using (a) linear interpolation and (b) last observation carried forward. To ensure insensitivity of the LME analysis of weights to missing values, the analysis was repeated on each of the data treated using these imputation methods. Alternatively,



**Fig. 1 – Study flowchart.** Thirteen individuals in the diabetic group and 6 in the control group did not complete the program ( $P = .11$ ); noncompleters were more likely to be female (female:  $n = 17$  vs male:  $n = 2$ ,  $P = .015$ ). Overall duration within the study was less in the diabetic group compared with controls (last contact:  $17.8 \pm 1.5$  vs  $21.8 \pm 0.9$  weeks,  $P = .027$ ), as withdrawal occurred earlier in the diabetic group ( $6.5 \pm 1.7$  vs  $12.0 \pm 1.8$  weeks,  $P = .044$ ). Age and BMI were not different for dropouts vs completers; and the diabetic and control dropouts did not differ in age, sex, or baseline BMI. Reasons for withdrawal were logistical ( $n = 6$ ), psychosocial ( $n = 3$ ), financial ( $n = 2$ ), diet intolerance ( $n = 1$ ), or no reason given ( $n = 7$ ).



**Table 1 – Baseline characteristics for subjects who completed the study (n = 51)**

	Diabetes (n = 24)	Control (n = 27)	P
Age (y)	54.4 ± 1.5	48.5 ± 2.2	.035
Sex (n [% male])	12 (50)	9 (33)	.23
Duration of diabetes (y)	7.8 ± 1.4	–	–
Cardiovascular disease (n [%])	4 (13)	3 (11)	.69
Diet or metformin therapy alone (n)	13	–	–
Sulfonylurea or insulin therapy (n insulin [n])	11 (6)	–	–
ACEi-ARB therapy (n [%])	16 (67)	4 (15)	<.001
Statin therapy (n [%])	17 (71)	6 (22)	<.001
Systolic blood pressure (mm Hg)	138 ± 3	132 ± 3	.15
Diastolic blood pressure (mm Hg)	82 ± 2.5	80 ± 2	.51
HbA <sub>1c</sub> (%)	8.0 ± 0.3	5.7 ± 0.1	<.001
Fasting plasma glucose (mmol/L)	9.5 ± 0.5	5.5 ± 0.1	<.001
Fasting plasma insulin (pmol/L)	239 ± 46	99 ± 9	.007
Fasting plasma C-peptide (nmol/L)	1.32 ± 0.16	1.14 ± 0.08	.32
Total cholesterol (mmol/L)	4.5 ± 0.3	5.5 ± 0.2	.011
Triglycerides (mmol/L)	2.7 ± 0.7	1.6 ± 0.2	.15
HDL cholesterol (mmol/L)	1.00 ± 0.05	1.25 ± 0.08	.012
LDL cholesterol (mmol/L)	2.4 ± 0.2	3.6 ± 0.2	<.001
C-reactive protein (mg/L)	7.1 ± 1.0	5.7 ± 1.0	.35
Creatinine (μmol/L)	74 ± 6	70 ± 3	.56
Thyrotropin (mIU/L)	2.5 ± 0.5	2.2 ± 0.2	.51
Sex hormone binding globulin (nmol/L)	33 ± 4	46 ± 5	.041

Continuous variables were analyzed by Student unpaired t test; and categorical data, by the  $\chi^2$  test or Fisher exact test. Continuous data expressed as mean ± SEM. ACEi indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin-2 receptor blocker; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

for parameters varying with a likelihood of early peak concentrations, including insulin and  $\beta$ -hydroxybutyrate, the LME analysis was performed on raw data only to avoid large bias caused by interpolation. For analysis of change in weight and body composition, to adjust for differences between the groups, an analysis of covariance (ANCOVA) was undertaken, centering weight change and age and including the grouping factor and weight change–group interaction. Statistical significance was assigned at the  $P < .05$  level. Correlations are reported as Pearson correlation coefficients. R version 2.10.0 (The R Foundation for Statistical Computing, Vienna, Austria) and Minitab statistical software version 16 (State College, PA) were used for the analyses.

### 3. Results

The baseline characteristics of the 70 subjects recruited were similar to the characteristics of the 51 subjects who completed the study (Table 1). The diabetic group was older with higher HbA<sub>1c</sub>, fasting glucose, and fasting insulin concentrations; and a greater proportion was on antihypertensive and lipid-lowering therapies. Although the diabetic and control groups were of similar weight at baseline (Table 2), the diabetic group had greater waist circumference ( $P = .009$ ) and waist-to-hip ratio ( $P = .006$ ). Compared with controls at baseline, FMI was not different at baseline ( $P = .34$ ), whereas lean tissue mass ( $P = .018$ ) and LTMI ( $P = .019$ ) were higher in the diabetic group.

#### 3.1. Diabetes vs control: weight loss

Weight loss at week 24 was not different between the diabetic ( $n = 37$ ) and control ( $n = 33$ ) groups (ITT:  $5.5 \pm 1.1$  vs  $7.7 \pm 1.2$  kg,  $P = .18$ ; PP:  $8.5 \pm 1.3$  vs  $9.4 \pm 1.2$  kg,  $P = .64$ ; Fig. 2). No differences were seen at any other time points, and weight nadir was also similar in the diabetic vs the control group (ITT:  $8.0 \pm 1.1$  vs  $10.1 \pm 0.9$  kg,  $P = .14$ ; PP:  $11.2 \pm 1.2$  vs  $11.2 \pm 1.0$  kg,  $P = .96$ ). Greater than 5% maximum weight loss was achieved by 25 of 37 diabetic subjects and 26 of 33 controls (68% vs 79%,  $P = .29$ ), whereas greater than 10% weight loss was achieved by 9 diabetic and 14 control subjects (24% vs 42%,  $P = .11$ ). Percentage weight lost from baseline at week 24 also revealed no significant differences between the diabetic and control groups (ITT:  $5.1\% \pm 1.0\%$  vs  $7.5\% \pm 1.2\%$ ,  $P = .13$ ; PP:  $7.8\% \pm 1.2\%$  vs  $9.2\% \pm 1.2\%$ ,  $P = .45$ ).

Adjusting for age and sex, the LME analysis of weight over time indicated that group (control or diabetic) was not significant ( $P = .20$ , ITT;  $P = .20$ , PP), whereas time was highly significant ( $P < .001$ ). No significant interaction was detected between time and group. For the study group as a whole, men lost more weight than women in both the ITT ( $P = .018$ ) and PP ( $P = .007$ ) analyses. The LME analysis was insensitive to within-study missing values, with all  $P$  values reported from the linear interpolation and last observation carried forward approaches (for both ITT and PP) being identical to 2 or 3 decimal places to those reported above.

#### 3.2. Diabetes vs control: change in body composition

Both groups lost significant amounts of lean and adipose tissue, including central fat, with the greater baseline lean tissue mass and LTMI in the diabetic group persisting at the end of the study (Table 2). In the 51 subjects who completed PP, change in BMI was a significant predictor for change in FMI (Fig. 3A and Table 3,  $P < .001$ ). However, the association between change in BMI and FMI was different between the control and diabetic groups (Fig. 3A), with greater decreases in BMI expected to result in smaller decreases in FMI in the diabetic group (diabetes: 0.574 decrease per unit decrease in BMI vs controls: 0.905 decrease per unit decrease in BMI,  $P = .003$ ). The findings persisted after adjusting for various baseline measures (Table 3). Akaike information criterion (AIC) model selection included baseline adjustments for BMI, FMI, and LTMI that resulted in a very good model (adjusted  $R^2 = 0.881$ ).

In the 51 subjects, weight change correlated with change in android-region fat ( $r = 0.869$ ,  $P < .001$ ). Absolute changes in android-region fat were similar in both groups ( $P = .71$ ), with no difference seen after adjusting for age and sex. By contrast, the diabetic group lost less gynoid-region fat mass ( $0.084$  vs  $0.146$ ,  $P = .001$ ) per unit weight loss, which persisted after adjusting for age and sex ( $P = .023$ ). A difference between sexes was seen, with men losing less gynoid-region fat than women ( $0.341$  less per unit weight loss when adjusting for the other variables,  $P = .002$ ).

Change in weight correlated with change in lean tissue mass in the 51 completers ( $r = 0.458$ ,  $P = .001$ ). Change in BMI did not predict change in LTMI (Fig. 3B and Table 3,  $P = .20$ ), and the association between changes in BMI and LTMI was not different between the control and diabetic groups ( $P = .99$ ). The

**Table 2 – Effect of VLCD in diabetic and control subjects**

	Diabetes (n = 24)			Control (n = 27)		P value for change: diabetes vs control	
Clinical	Baseline	End	Change	Baseline	End	Change	
Weight (kg)	108.9 ± 3.1	100.4 ± 3.1	–8.5 ± 1.3 <sup>§</sup>	103.2 ± 3.5	93.8 ± 3.6	–9.4 ± 1.2 <sup>§</sup>	0.64 (0.52)
BMI (kg/m <sup>2</sup> )	38.1 ± 1.0	35.2 ± 1.1	–3.0 ± 0.5 <sup>§</sup>	36.6 ± 1.1	33.3 ± 1.2	–3.3 ± 0.4 <sup>§</sup>	0.58 (0.56)
Waist circumference (cm)	122.0 ± 2.0 <sup>†</sup>	114.0 ± 2.0	–8.0 ± 1.0 <sup>§</sup>	113.0 ± 2.5	106.0 ± 3.0	–7.2 ± 1.1 <sup>§</sup>	0.62 (0.65)
Waist-to-hip ratio	0.99 ± 0.02 <sup>†</sup>	0.96 ± 0.02	–0.03 ± 0.01 <sup>†</sup>	0.92 ± 0.02	0.91 ± 0.02	–0.01 ± 0.01 <sup>*</sup>	0.14 (0.51)
BIA							
Estimated RMR (kcal/d)	1609 ± 69	1572 ± 63	–37 ± 10 <sup>†</sup>	1551 ± 58	1515 ± 51	–52 ± 11 <sup>§</sup>	0.33 (0.15)
Biochemical							
Estimated total body protein <sup>a</sup> (kg)	13.7 ± 0.6	12.9 ± 0.6	0.8 ± 0.2 <sup>§</sup>	13.2 ± 0.5	12.1 ± 0.5	1.0 ± 0.2 <sup>§</sup>	0.40 (0.32)
DXA							
Lean tissue (kg)	60.7 ± 2.7 <sup>†</sup>	58.4 ± 2.6	–2.2 ± 0.6 <sup>†</sup>	52.0 ± 2.3	50.1 ± 2.2	–1.9 ± 0.5 <sup>†</sup>	0.73 (0.47)
Appendicular LTMI (kg/m <sup>2</sup> )	8.8 ± 0.3 <sup>†</sup>	8.4 ± 0.3	–0.4 ± 0.1 <sup>†</sup>	7.9 ± 0.3	7.6 ± 0.3	–0.2 ± 0.1 <sup>†</sup>	0.33 (0.67)
Total adipose tissue (kg)	42.7 ± 2.0	37.8 ± 2.2	–5.5 ± 0.8 <sup>§</sup>	45.9 ± 2.0	38.6 ± 2.2	–6.8 ± 1.2 <sup>§</sup>	0.35 (0.40)
FMI (kg/m <sup>2</sup> )	15.2 ± 0.9	13.6 ± 1.0	–1.9 ± 0.3 <sup>§</sup>	16.4 ± 0.8	13.7 ± 0.8	–2.4 ± 0.5 <sup>§</sup>	0.36 (0.40)
Adipose tissue, android (kg)	4.8 ± 0.2	4.1 ± 0.2	–0.8 ± 0.1 <sup>§</sup>	4.7 ± 0.2	3.9 ± 0.3	–0.8 ± 0.1 <sup>§</sup>	0.71 (0.74)
Adipose tissue, gynoid (kg)	6.6 ± 0.4	5.9 ± 0.4	–0.9 ± 0.1 <sup>§</sup>	7.6 ± 0.4	6.5 ± 0.4	–1.1 ± 0.2 <sup>§</sup>	0.34 (0.58)

Data expressed as mean ± SEM. Comparisons of changes for the diabetic and control groups were analyzed by both unpaired t test and ANCOVA adjusting for baseline measurements and are reported in the right-hand column (ANCOVA P value in parentheses). BIA indicates bioimpedance analysis; RMR, resting metabolic rate.

\* P < .1, † P < .05, ‡ P < .01, § P < .001 for diabetic vs control group comparisons at baseline, analyzed by unpaired t test and reported in “Diabetes/Baseline” column, and intragroup comparisons of change, analyzed by Student paired t test and reported in “Change” columns.

<sup>a</sup> Total body protein estimated using height, weight, and daily urinary creatinine excretion.

AIC model selection including baseline adjustments resulted in a model that was weaker than for FMI ( $R^2_{\text{adj}} = 0.205$ ). Estimated total body protein decreased in both groups, but no difference was seen between the diabetic and control subjects. The percentage of weight lost that was fat-free mass was also not different between groups (diabetes: 32% ± 9% vs control: 25% ± 6%,  $P = .53$ ).

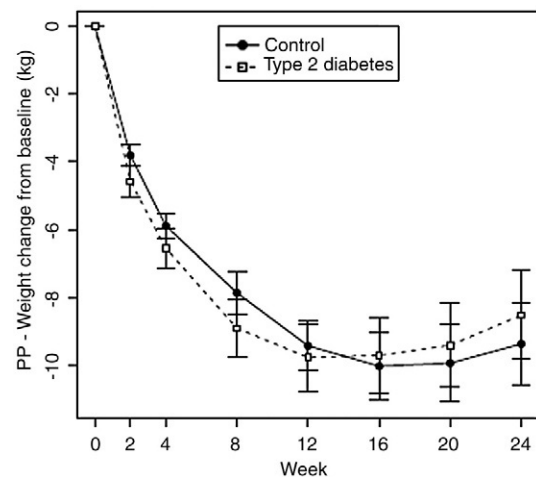
### 3.3. Diabetes vs control: change in ketosis, insulin concentrations, and hunger score

Fasting plasma insulin concentrations were decreased at week 2 compared with baseline in both diabetic and control subjects (both  $P < .001$ ) and remained significantly lower than baseline values in the diabetic group at all time points to week 24 inclusive (all  $P < .01$ , Fig. 4A). However, compared with controls, insulin concentrations in the diabetic group were significantly higher at baseline and each time point to week 24 inclusive (all  $P < .05$ ). Overall, the LME model showed that a decrease in weight was associated with decreases in insulin concentration ( $P < .001$ ).

Fasting  $\beta$ -hydroxybutyrate concentrations were significantly elevated from baseline at weeks 2 and 4 (both  $P < .001$ ) and week 8 ( $P = .046$ ) in the diabetic group and at week 2 through to week 16 inclusive (weeks 2 and 4: both  $P < .001$ ; week 8:  $P = .002$ ; weeks 12 and 16: both  $P < .05$ ) in the control group (Fig. 4B). The LME model found lower  $\beta$ -hydroxybutyrate concentrations in the diabetic group compared with controls at week 2 ( $P = .013$ ). A significantly lower peak fasting plasma  $\beta$ -hydroxybutyrate concentration in the diabetic group was observed:  $0.29 \pm 0.03$  mmol/L (diabetic) vs  $0.43 \pm 0.04$  mmol/L (control),  $P = .009$ . Considering completers only, there was a trend toward significance:  $0.35 \pm 0.04$  mmol/L (diabetic) vs  $0.46 \pm 0.05$  mmol/L (control),  $P = .080$ . Peak  $\beta$ -hydroxybutyrate

concentration correlated with change in weight at the end of the intensive phase at week 12 ( $r = -0.465$ ,  $P < .001$ ) and week 24 ( $r = -0.387$ ,  $P = .005$ ).

No significant difference was observed in visual analogue scale hunger scores between the 2 groups (all  $P > .05$ ). There was no correlation between overall hunger and satiety scores and weight loss, ketone concentrations, or plasma insulin concentrations in either group or subgroup. No correlation was found between change in urinary glucose or calorie loss and change in body weight in the diabetic group (data not shown). Self-reported duration of exercise was not different between groups



**Fig. 2 – Change in weight by PP analysis in the diabetic (□) and control (●) groups (n = 51). Data are shown as mean ± SEM. Linear mixed effects modeling for weight: the fixed effect time was significant ( $P < .001$ ), whereas group and time-group interaction were not ( $P = .23$  and  $P = .54$ , respectively).**

(diabetic group:  $10 \pm 2$  min/d vs control group:  $16 \pm 2$  min/d,  $P = .14$ ) and was much less than the quantity recommended to subjects upon commencement of the program.

### 3.4. Subgroup analysis by diabetic treatment

No difference in weight loss was seen at 24 weeks between the diabetic SUI subgroup ( $n = 17$ ) compared with the DMF subgroup ( $n = 20$ ) or control group by ITT or PP analysis (PP:  $8.4 \pm 2.1$  kg SUI [ $n = 11$ ] vs  $8.6 \pm 1.7$  kg DMF [ $n = 13$ ] vs  $9.4 \pm 1.2$  kg

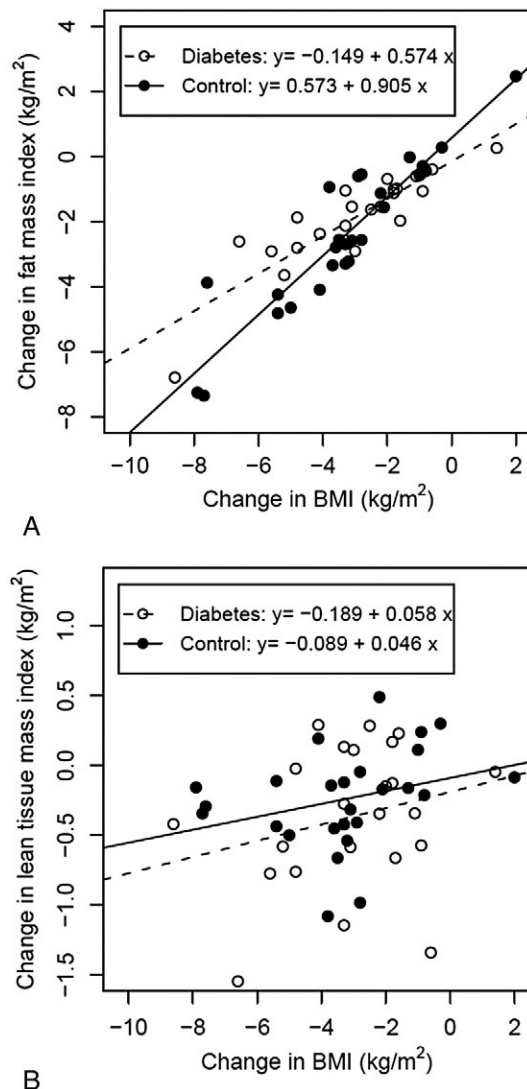
control,  $P = .89$ ). Furthermore, the LME analysis, controlling for age and sex, did not reveal significant differences in weight change between subgroups ( $P = .23$ ), including no significant interaction between subgroup and time ( $P = .97$ ), indicating similar rates of change in weight in the control group and DMF and SUI subgroups throughout the study. At no time point were significant differences in mean weight detected between any of the subgroups (Tukey test: all  $P > .20$ ).

Contrastingly, compared with controls, the SUI subgroup experienced smaller reductions in FMI per unit decrease in BMI (SUI: 0.432 decrease per unit decrease in BMI vs controls: 0.905 decrease per unit decrease in BMI,  $P < .001$ ). This difference did not reach statistical significance when comparing the DMF subgroup to controls (DMF: 0.697 decrease per unit decrease in BMI vs controls: 0.905 decrease per unit decrease in BMI,  $P = .12$ ); the comparison between the SUI and DMF subgroups also was not significant ( $P = .10$ ).

A similar analysis for change in LTMI and BMI revealed that neither of the diabetic subgroups was significantly different than the controls. However, a significant difference was found between the SUI and DMF subgroups: 0.151 decrease per unit decrease in BMI (SUI) vs 0.032 increase per unit decrease in BMI (DMF),  $P = .030$ . The increase in LTMI relative to the decrease in BMI in the DMF subgroup was not significant ( $P = .58$ ).

Compared with controls, the SUI subgroup had increased plasma insulin concentrations at each time point (all  $P < .030$ ). The LME analysis indicated a difference between subgroups in the pattern of insulin concentration change relative to the starting baseline concentration, with time ( $P < .001$ ), subgroup ( $P = .001$ ), and time-subgroup interaction ( $P < .001$ ) all significant. Differences in rates of change in insulin over time were specifically due to differences between the SUI subgroup and controls. By contrast, the DMF subgroup had similar insulin concentrations to controls throughout the study; and concentrations were significantly lower when compared with the SUI subgroup at nearly all time points (all  $P < .03$ , except for week 20:  $P = .068$ ). The LME analysis of plasma  $\beta$ -hydroxybutyrate concentrations revealed lower concentrations at week 2 in the DMF ( $P = .048$ ) and the SUI ( $P = .037$ ) subgroups compared with controls. The LME analysis did not reveal any significant differences in plasma  $\beta$ -hydroxybutyrate concentration between the DMF and SUI subgroups at any time point, including no difference in the rates of change over time.

No significant episodes of hypoglycemia occurred in the SUI subgroup, and quantity of exercise did not differ across subgroups.



**Fig. 3 – Change in fat and lean tissue vs weight loss over 24 weeks in the diabetic (○) and control (●) groups ( $n = 51$ ). A, Correlations between change in BMI and change in FMI (fat mass [kilograms]/height<sup>2</sup> [square meter]): all completers:  $r = 0.887$ , diabetes:  $r = 0.878$ , controls:  $r = 0.920$ ; all  $P < .001$ . ANCOVA adjusted  $R^2$ : total cohort:  $R^2_{\text{adj}} = 0.82$ ; group specific: diabetes— $R^2_{\text{adj}} = 0.76$ , controls— $R^2_{\text{adj}} = 0.84$ . B, Correlations between change in BMI and change in appendicular LTMI (kilograms per square meter), a skeletal muscle mass surrogate: diabetes:  $r = 0.248$ ,  $P = .25$ ; controls:  $r = 0.298$ ,  $P = .14$ .**

## 4. Discussion

We report that weight loss in obese individuals with T2DM is not different to that of individuals without diabetes completing a 24-week VLCD program, although reduction in fat mass per unit weight loss is less. To our knowledge, this is the largest comparative study of VLCDs performed in diabetic and nondiabetic subjects and the only comparative study evaluating change in DXA-measured body composition in diabetic

**Table 3 – Three estimated models for change in fat and lean tissue mass indices and associated R<sup>2</sup>**

Model type	Estimated model for $\Delta$ FMI	R <sup>2</sup> <sub>adj</sub>	Model type	Estimated model for $\Delta$ LTMI	R <sup>2</sup> <sub>adj</sub>
Simple ANCOVA (Fig. 3)	$\Delta$ FMI = 0.573 <sup>†</sup> + 0.905 <sup>§</sup> ( $\Delta$ BMI) – 0.722 <sup>*</sup> T2DM – 0.330 <sup>†</sup> (T2DM $\times$ $\Delta$ BMI)	0.819	Simple ANCOVA (Fig. 3)	$\Delta$ LTMI = –0.089 + 0.046 ( $\Delta$ BMI) – 0.100 T2DM + 0.012 (T2DM $\times$ $\Delta$ BMI)	0.030
ANCOVA with baseline adjustments	$\Delta$ FMI = –1.497 + 0.820 <sup>§</sup> ( $\Delta$ BMI) – 0.873 <sup>†</sup> T2DM – 0.007 age – 0.266 male + 0.301 <sup>†</sup> BMI – 0.354 <sup>†</sup> FMI – 0.374 <sup>†</sup> LTMI – 0.237 <sup>†</sup> (T2DM $\times$ $\Delta$ BMI)	0.877	ANCOVA with baseline adjustments	$\Delta$ LTMI = 1.421 + 0.028 ( $\Delta$ BMI) – 0.042 T2DM – 0.014 <sup>*</sup> age – 0.054 male + 0.137 <sup>†</sup> BMI – 0.170 <sup>†</sup> FMI – 0.393 <sup>†</sup> LTMI + 0.016 (T2DM $\times$ $\Delta$ BMI)	0.155
ANCOVA with baseline adjustments and AIC selection	$\Delta$ FMI = –1.882 <sup>†</sup> + 0.826 <sup>§</sup> ( $\Delta$ BMI) – 0.838 <sup>†</sup> T2DM + 0.254 <sup>§</sup> BMI – 0.280 <sup>†</sup> FMI – 0.315 <sup>*</sup> LTMI – 0.231 <sup>†</sup> (T2DM $\times$ $\Delta$ BMI)	0.881	ANCOVA with baseline adjustments and AIC selection	$\Delta$ LTMI = 1.599 <sup>†</sup> + 0.037 ( $\Delta$ BMI) – 0.015 <sup>†</sup> age + 0.128 <sup>†</sup> BMI – 0.156 <sup>†</sup> FMI – 0.395 <sup>†</sup> LTMI	0.205
$\Delta$ indicates change. <sup>*</sup> P < .1. <sup>†</sup> P < .05. <sup>‡</sup> P < .01. <sup>§</sup> P < .001.					

vs nondiabetic subjects. Earlier comparative studies suggested a reduction in the efficacy of VLCDs in diabetic subjects [12,13]; however, these studies had important group differences at baseline and were smaller and of shorter duration than the present study.

One possible explanation for the finding of decreased fat mass reduction per unit weight loss is the anabolic effect of higher circulating concentration of insulin in diabetic subjects, which could promote adipose tissue storage [30]. Our subgroup analysis, albeit in small numbers of subjects, found that diabetic subjects on sulfonylurea or insulin therapy, who had persistently greater plasma insulin concentrations, had a reduction in fat loss per unit decrease in weight compared with the control group. However, interestingly, a similar trend was seen in the diet or metformin subgroup, in which plasma insulin concentrations were not different to the control group. Thus, factors other than hyperinsulinemia, including possible altered circulating adipokines in the diabetic group, may also play a role in modulating adipose tissue loss in response to VLCDs.

The observation of similar weight loss for less fat reduction in diabetic compared with control subjects implies that loss of fat-free mass must be greater in the diabetic group. However, importantly, we found similar reductions in both groups in estimated total body protein and appendicular lean tissue mass, an estimate of skeletal muscle mass. Therefore, greater loss of body water in the diabetic group, possibly related to a decrease in sodium retention mediated by hyperinsulinemia or sympathetic nervous system overdrive [31] or greater reductions in glycogen storage [32], might explain differences between groups in change in body composition. The relative preservation of skeletal muscle mass in both groups may have been modulated by an increase in ketogenesis in the early phase of the diet, as ketosis has been shown to preserve lean muscle mass by reducing amino acid release from muscle as well as decreasing hepatic gluconeogenesis from amino acids [33]. Theoretically, in diabetic subjects, higher circulating insulin concentrations could inhibit ketosis, allowing greater muscle catabolism to occur. However, higher insulin concentrations may offset this effect by directly inhibiting proteolysis [34]. We found the VLCD-induced rise in  $\beta$ -hydroxybutyrate was attenuated in the diabetic group, including in the subgroup on diet or metformin therapy alone, in which no

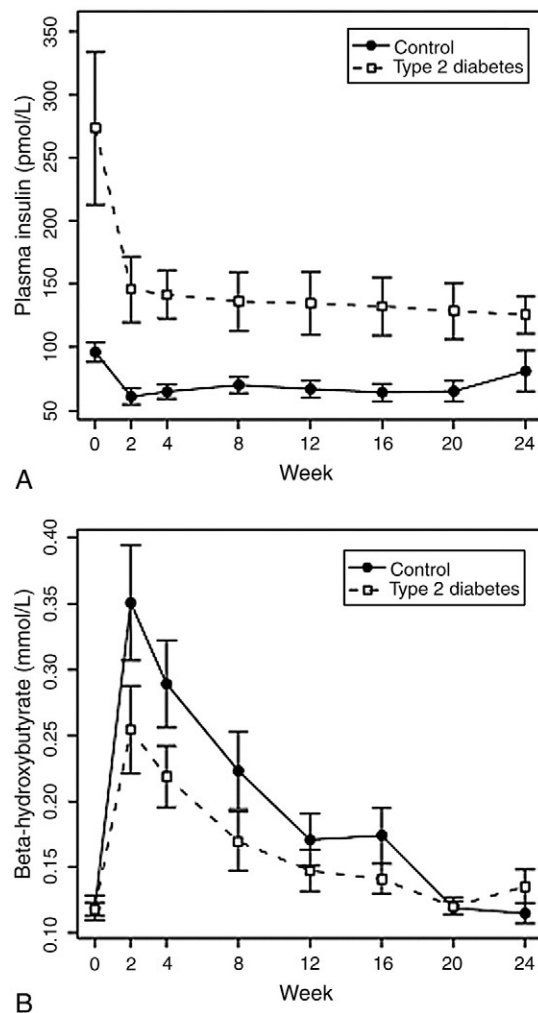
decrease in LTMI was observed. Therefore, ketosis in this setting may play only a minor role in the preservation of lean tissue, with other factors, including hormonal (eg, growth hormone) changes, possibly involved [35].

Overall, our data do suggest that ketosis has an important role in VLCD efficacy, demonstrated by the strong relationship between peak  $\beta$ -hydroxybutyrate concentrations and reduction in weight at weeks 12 and 24. Although evidence exists to support the appetite-suppressing effects of ketone bodies [36], exogenous insulin treatment and hyperinsulinemia are, conversely, associated with increased appetite [37]. In our study, despite differences in circulating insulin and  $\beta$ -hydroxybutyrate concentrations, we were unable to show a difference between the diabetic and control groups in hunger and satiety using a visual analogue scale. Furthermore, we observed similar changes in ketosis in both diabetic subgroups compared with controls, despite higher insulin concentrations being found only in the diabetic subgroup on sulfonylurea/insulin treatment. Thus, it remains uncertain that hyperinsulinemia is the key inhibitor of ketogenesis in diabetic patients on VLCDs; rather, factors such as inconsistent diet adherence (possibly related to attempts to avoid hypoglycemia) may be more important.

In accord with the few extant studies [14,15,38], we report that men lost more weight than women during this VLCD program. More women than men did not complete the program, but there were no differences in women compared with men in ketosis or hunger to explain this finding. Of note, both men and women who withdrew were losing less weight and had lower ketone concentrations than those who persisted, suggesting poor diet adherence before dropout.

Compared with the 13- to 15-kg weight loss at 12 to 24 weeks reported in earlier studies of T2DM subjects undergoing VLCDs [9,39,40], the mean weight loss in our study was somewhat less. This may reflect a “calendar effect,” whereby current environmental or other factors make weight loss more difficult than in times past. Although baseline characteristics including age, sex, BMI, diabetic control, and therapies were similar in our cohort to previous studies, it remains possible that our study population represents a more difficult to treat group than those studied previously. Notably, most of our subjects were established patients in the diabetes or weight





**Fig. 4 – Change in plasma insulin (A) and ketosis (B) in the diabetic (□) and control (●) groups. Data are shown as mean  $\pm$  SEM. Insulin: LME modeling revealed that the fixed effects time and group were significant ( $P < .001$  and  $P = .002$ ) and also time-group interaction ( $P = .006$ ). Covariate weight was also significant ( $P < .001$ ), with decreases in weight associated with decreases in insulin concentration.  $\beta$ -Hydroxybutyrate concentrations: LME modeling showed that the fixed effect time was significant ( $P < .001$ ), but not the group ( $P = .11$ ) or time-group interaction ( $P = .34$ ). Covariate weight was not significant ( $P = .24$ ).**

control clinics at our tertiary referral center rather than patients managed in primary care settings.

This study has some limitations. Firstly, in designing the study, based on the findings of Wing et al [3], we expected a 5-kg absolute difference in weight loss between groups and so powered our study to detect this difference. Thus, we cannot exclude the possibility of a smaller difference in weight change in diabetic compared with nondiabetic individuals, although any difference of this magnitude is unlikely to be clinically significant, particularly in the intermediate and longer term. Importantly, our finding of comparable weight loss in diabetic and control subjects is supported by both the statistical modeling and the near-identical weight change in

both groups recorded during the study, as shown in Fig. 2. Secondly, as in other VLCD studies, direct measurement of activity levels (eg, pedometry) over the 24 weeks was not performed; and so we relied on subject reportage of time spent exercising. An earlier VLCD study suggested that exercise does not increase initial weight loss to 16 weeks [41], although exercise may have a role in the maintenance of weight loss achieved during the initial VLCD intervention. We found that mean reported time in exercise in both groups was less than half of our recommendation of 150 min/wk, with no difference between groups identified. Thirdly, our study was undertaken in a tertiary hospital ambulatory care setting with participants largely drawn from specialist clinics; therefore, the findings may not be applicable to VLCD programs in other settings. Finally, as all participants underwent the intervention, this was not a randomized trial; and therefore, the possibility of unrecognized confounding factors cannot be eliminated.

In summary, contrary to early studies, obese individuals with T2DM lost similar amounts of weight compared with sex- and weight-matched obese individuals without diabetes completing a 24-week VLCD program. However, for an equivalent reduction in BMI, the reduction in FMI was less in subjects with diabetes vs controls. This observation was most pronounced in diabetic subjects on insulin and sulfonylurea therapy; however, a similar trend was suggested in diabetic individuals only on dietary therapy or metformin. Reduction in appendicular LTM—a surrogate of skeletal muscle mass—and estimated total body protein was not different between the diabetic and control groups. The finding that obese individuals with T2DM need to lose greater weight for equivalent reductions in fat compared with nondiabetic obese individuals may have implications not only for dietary weight loss interventions but also for pharmacotherapeutic and surgical treatments. Further investigation into mechanisms underlying the differences in body composition in obese subjects with T2DM compared with nondiabetic subjects undergoing weight loss interventions, including neuroendocrine differences and altered adipokine signaling, is warranted.

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## Conflict of Interest

During the study period, JP was Chair of the Optifast Medical Advisory Committee for Nestlé Nutrition. STB has received a training scholarship from the National Health and Medical Research Council of Australia. JP is supported by the Sir Edward Dunlop Medical Research Foundation. No other conflicts of interest are identified.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at [doi:10.1016/j.metabol.2011.10.017](https://doi.org/10.1016/j.metabol.2011.10.017).

## REFERENCES

- [1] Nyenwe EA, Jerkins TW, Umpierrez GE, et al. Management of type 2 diabetes: evolving strategies for the treatment of patients with type 2 diabetes. *Metabolism* 2011;60:1-23.
- [2] American Diabetes Association. Standards of medical care in diabetes—2011. *Diabetes Care* 2011;34(Suppl 1):S11-61.
- [3] Wing RR, Marcus MD, Epstein LH, et al. Type II diabetic subjects lose less weight than their overweight nondiabetic spouses. *Diabetes Care* 1987;10:563-6.
- [4] Khan MA, St Peter JV, Breen GA, et al. Diabetes disease stage predicts weight loss outcomes with long-term appetite suppressants. *Obes Res* 2000;8:43-8.
- [5] Gokcel A, Karakose H, Ertorer EM, et al. Effects of sibutramine in obese female subjects with type 2 diabetes and poor blood glucose control. *Diabetes Care* 2001;24:1957-60.
- [6] Scheen AJ, Finer N, Hollander P, et al. Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. *Lancet* 2006;368:1660-72.
- [7] Campos GM, Rabl C, Mulligan K, et al. Factors associated with weight loss after gastric bypass. *Arch Surg* 2008;143:877-83 [discussion 884].
- [8] Guare JC, Wing RR, Grant A. Comparison of obese NIDDM and nondiabetic women: short- and long-term weight loss. *Obes Res* 1995;3:329-35.
- [9] Capstick F, Brooks BA, Burns CM, et al. Very low calorie diet (VLCD): a useful alternative in the treatment of the obese NIDDM patient. *Diabetes Res Clin Pract* 1997;36:105-11.
- [10] Dhindsa P, Scott AR, Donnelly R. Metabolic and cardiovascular effects of very-low-calorie diet therapy in obese patients with type 2 diabetes in secondary failure: outcomes after 1 year. *Diabet Med* 2003;20:319-24.
- [11] Wing RR. Use of very-low-calorie diets in the treatment of obese persons with non-insulin-dependent diabetes mellitus. *J Am Diet Assoc* 1995;95:569-72 [quiz 573-64].
- [12] Henry RR, Wiest-Kent TA, Scheaffer L, et al. Metabolic consequences of very-low-calorie diet therapy in obese non-insulin-dependent diabetic and nondiabetic subjects. *Diabetes* 1986;35:155-64.
- [13] Harris MD, Davidson MB, Bush MA. Exogenous insulin therapy slows weight loss in type 2 diabetic patients. *Int J Obes* 1988;12:149-55.
- [14] Torgerson JS, Lissner L, Lindroos AK, et al. VLCD plus dietary and behavioural support versus support alone in the treatment of severe obesity. A randomised two-year clinical trial. *Int J Obes Relat Metab Disord* 1997;21:987-94.
- [15] Mustajoki P, Pekkarinen T. Very low energy diets in the treatment of obesity. *Obes Rev* 2001;2:61-72.
- [16] Foster GD, Wadden TA, Feurer ID, et al. Controlled trial of the metabolic effects of a very-low-calorie diet: short- and long-term effects. *Am J Clin Nutr* 1990;51:167-72.
- [17] Foster GD, Wadden TA, Peterson FJ, et al. A controlled comparison of three very-low-calorie diets: effects on weight, body composition, and symptoms. *Am J Clin Nutr* 1992;55:811-7.
- [18] Donnelly JE, Pronk NP, Jacobsen DJ, et al. Effects of a very-low-calorie diet and physical-training regimens on body composition and resting metabolic rate in obese females. *Am J Clin Nutr* 1991;54:56-61.
- [19] Burgess NS. Effect of a very-low-calorie diet on body composition and resting metabolic rate in obese men and women. *J Am Diet Assoc* 1991;91:430-4.
- [20] Randle PJ, Garland PB, Hales CN, et al. The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet* 1963;1:785-9.
- [21] Delbridge E, Proietto J. State of the science: VLED (very low energy diet) for obesity. *Asia Pac J Clin Nutr* 2006;15(Suppl):49-54.
- [22] Baker S, Jerums G, Proietto J. Effects and clinical potential of very-low-calorie diets (VLCDs) in type 2 diabetes. *Diabetes Res Clin Pract* 2009;85:235-42.
- [23] Noakes M, Clifton P. The CSIRO total wellbeing diet. Melbourne: Penguin, Australia; 2005.
- [24] Stroud DB, Borovnicar DJ, Lambert JR, et al. Clinical studies of total body nitrogen in an Australian hospital. *Basic Life Sci* 1990;55:177-82.
- [25] Wang Z, Stroud DB, Heymsfield SB, et al. Potassium-related total body protein and body cell mass models: cross-validation studies in patients with chronic diseases. *Int J Body Compos Res* 1994;2:141-8.
- [26] Borovnicar DJ, Wong KC, Kerr PG, et al. Total body protein status assessed by different estimates of fat-free mass in adult peritoneal dialysis patients. *Eur J Clin Nutr* 1996;50:607-16.
- [27] Chearskul S, Delbridge E, Shulkes A, et al. Effect of weight loss and ketosis on postprandial cholecystokinin and free fatty acid concentrations. *Am J Clin Nutr* 2008;87:1238-46.
- [28] Mazess RB, Barden HS, Bisek JP, et al. Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. *Am J Clin Nutr* 1990;51:1106-12.
- [29] Kim J, Wang Z, Heymsfield SB, et al. Total-body skeletal muscle mass: estimation by a new dual-energy x-ray absorptiometry method. *Am J Clin Nutr* 2002;76:378-83.
- [30] Hales CN, Randle PJ. Effects of low-carbohydrate diet and diabetes mellitus on plasma concentrations of glucose, non-esterified fatty acid, and insulin during oral glucose-tolerance tests. *Lancet* 1963;1:790-4.
- [31] Peters A, Schweiger U, Fruhwald-Schultes B, et al. The neuroendocrine control of glucose allocation. *Exp Clin Endocrinol Diabetes* 2002;110:199-211.
- [32] Henry RR, Gumbiner B. Benefits and limitations of very-low-calorie diet therapy in obese NIDDM. *Diabetes Care* 1991;14:802-23.
- [33] Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot Essent Fatty Acids* 2004;70:309-19.
- [34] Fryburg DA, Jahn LA, Hill SA, et al. Insulin and insulin-like growth factor-I enhance human skeletal muscle protein

- anabolism during hyperaminoacidemia by different mechanisms. *J Clin Invest* 1995;96:1722-9.
- [35] Volek JS, Sharman MJ, Love DM, et al. Body composition and hormonal responses to a carbohydrate-restricted diet. *Metabolism* 2002;51:864-70.
- [36] Pawan GL, Semple SJ. Effect of 3-hydroxybutyrate in obese subjects on very-low-energy diets and during therapeutic starvation. *Lancet* 1983;1:15-7.
- [37] Rodin J, Wack J, Ferrannini E, et al. Effect of insulin and glucose on feeding behavior. *Metabolism* 1985;34:826-31.
- [38] Pekkarinen T, Mustajoki P. Comparison of behavior therapy with and without very-low-energy diet in the treatment of morbid obesity. A 5-year outcome. *Arch Intern Med* 1997;157:1581-5.
- [39] Wing RR, Blair E, Marcus M, et al. Year-long weight loss treatment for obese patients with type II diabetes: does including an intermittent very-low-calorie diet improve outcome? *Am J Med* 1994;97:354-62.
- [40] Paisey RB, Harvey P, Rice S, et al. An intensive weight loss programme in established type 2 diabetes and controls: effects on weight and atherosclerosis risk factors at 1 year. *Diabet Med* 1998;15:73-9.
- [41] Sikand G, Kondo A, Foreyt JP, et al. Two-year follow-up of patients treated with a very-low-calorie diet and exercise training. *J Am Diet Assoc* 1988;88:487-8.