Calorie Restriction and Matched Weight Loss From Exercise: Independent and Additive Effects on Glucoregulation and the Incretin System in Overweight Women and Men

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OBJECTIVE

It is not known whether calorie restriction (CR) has additive benefits to those from exercise (EX)-induced weight loss. We hypothesized that weight loss from CR and EX (CREX) improves insulin sensitivity more than matched weight loss induced by EX or CR alone and that the incretin system may be involved in adaptations to CR.

RESEARCH DESIGN AND METHODS

Sedentary, overweight men and women (*n* = 52, 45–65 years of age) were randomized to undergo 6–8% weight loss by using CR, EX, or CREX. Glucose, insulin, C-peptide, insulin sensitivity, and incretin hormones (glucagon-like peptide 1 [GLP-1] and glucose-dependent insulinotropic polypeptide [GIP]) were measured during frequently sampled oral glucose tolerance tests (FSOGTTs). Incretin effects on insulin secretion were measured by comparing insulin secretion rates from the FSOGTTs to those from a glycemia-matched glucose infusion.

RESULTS

Despite similar weight losses in all groups, insulin sensitivity index values increased twofold more in the CREX group (2.09 ± 0.35 μM/kg/pM × 100) than in the CR (0.89 ± 0.39 μM/kg/pM × 100) and EX (1.04 ± 0.39 μM/kg/pM × 100) groups. Postprandial GLP-1 concentrations decreased only in the CR group (*P* = 0.04); GIP concentrations decreased in all groups. Incretin effects on insulin secretion were unchanged.

CONCLUSIONS

CR and EX have additive beneficial effects on glucoregulation. Furthermore, the adaptations to CR may involve reductions in postprandial GLP-1 concentrations. These findings underscore the importance of promoting both CR and EX for optimal health. However, because data from participants who withdrew from the study and from those who did not adhere to the intervention were excluded, the results may be limited to individuals who are capable of adhering to a healthy lifestyle intervention.

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Calorie restriction (CR) and exercise (EX) can lead to weight loss, and are effective for improving glucose tolerance and insulin action, and reducing type 2 diabetes risk (1–3). EX can improve glucoregulation by causing weight loss and by weight loss–independent effects, including increases in skeletal muscle GLUT4 transport protein levels (4) and greater insulin-mediated glucose disposal (5). In contrast, the beneficial effect of CR on glucoregulation is often attributed to weight loss alone. In this context, EX-induced weight loss would be expected to improve glucoregulation more than matched weight loss induced by CR. However, we (1) and others (6) have shown that EX-induced weight loss (without CR) does not provide greater improvements in glucoregulation than CR alone. A plausible explanation for this unexpected finding is that, in addition to providing benefits through weight loss, CR may also improve glucoregulation through other mechanisms. If this is true, then the combination of CR and EX (CREX) would be expected to improve glucoregulation more so than similar weight loss from EX alone; however, no studies have evaluated this possibility.

The purpose of the current study was to evaluate the hypothesis that CR and EX have additive effects, even in the absence of greater weight loss. We proposed that a 7% reduction in body mass induced by CREX results in greater improvements in glucose tolerance and insulin action than those resulting from similar weight loss induced by EX or CR alone. Another objective was to gain insights about unique mechanisms by which CR might alter glucoregulation (i.e., independent of weight loss induced by EX). Because the incretin system is a food-sensing system, we hypothesized that long-term restriction of food intake (i.e., CR), but not EX-induced weight loss, reduces postprandial incretin hormone levels while their actions to promote insulin secretion (i.e., incretin effects (7)) are maintained, suggesting enhanced pancreatic sensitivity to incretin hormones; this might be especially important for preventing the relative insulin deficiency that accompanies progression to type 2 diabetes. Furthermore, because one of the incretin hormones (glucagon-like peptide 1 [GLP-1]) promotes glucose uptake in muscle and adipose tissue (8,9), a reduction in GLP-1 with concomitant improvements in glycemic control would be suggestive of enhanced GLP-1 actions on glucose uptake.

RESEARCH DESIGN AND METHODS

Study Design and Randomization

Subjects were randomized, with stratification for sex, to CR, EX, or CREX, all of which were designed to induce a 6–8% weight loss. The initial allocation ratio of 1:1:1 was later revised to 2:2:1, with greater enrollment in the CR and EX groups to account for more withdrawals from these groups. Outcome measures were performed at baseline and after weight loss. Participants provided informed written consent to participate in the study, which was approved by the Institutional Review Boards at Saint Louis University and Washington University. The trial was registered at ClinicalTrials.gov (clinical trial reg. no. NCT00777621).

Participants

Overweight men and postmenopausal women (45–65 years of age, BMI 25.0–29.9 kg/m^2) were recruited from the St. Louis metropolitan area. Potential participants underwent screening, including a medical evaluation, and were excluded from the study if they had experienced a significant ($>$3%) weight change within 6 months and if they performed regular vigorous endurance EX (moderate to hard effort EX, $\geq$20 min/session, and three or more times per week). Other exclusion criteria were the presence of major chronic diseases, conditions that would interfere with EX or in which EX is contraindicated, or conditions that would interfere with interpretation of the results. Examples include diabetes (self-reported or fasting blood glucose level of $\geq$126 mg/dL), blood pressure of $\geq$160 mmHg systolic or $\geq$100 mmHg diastolic, musculoskeletal problems, and smoking. Use of glucoregulatory medications was also exclusionary. For other medications, participants were required to have been on stable dosages for $\geq$6 months prior to baseline testing and were advised to maintain dosages during the study.

Interventions

The interventions were designed to decrease body mass by 6–8% over 12–14 weeks. However, the intervention duration was adjusted as needed for participants to reach the weight loss goal. CR and EX prescriptions were based on estimates of baseline total energy expenditure (TEE) and energy intake, as follows: *1*) dietary reference intakes equations for estimated energy requirements (10); *2*) 3-day food diaries with nutrient analysis (described below); *3*) accelerometry (described below); and *4*) 7-day physical activity recalls (described below). Because energy intake and TEE are equal during weight stability, and because the participants were weight stable at baseline, the average of all four measures was used to reflect the TEE and energy intake. During the interventions, the prescriptions were adjusted as needed, with the goal of achieving weight loss at a rate of $\sim$0.5% per week. To eliminate the potentially confounding effects of negative energy balance on the results, body weight was stabilized by altering the CR and/or EX prescriptions for 2 weeks before follow-up testing, with the goal being to avoid weight changes of $>$0.5 kg, based on a 3-day rolling average weight. The participants recorded daily fasted morning body weight at home, and visited our clinic weekly to be weighed, turn in home weight logs, and undergo other intervention-specific requirements (described below).

CR

The CR intervention was designed to decrease energy intake by $\sim$20% without changing physical activity. During the initial 3 weeks and periodically thereafter, the participants completed 3-day food diaries that were used by the study dietitians for personalized dietary recommendations. The strategies for decreasing energy intake included food portion control and replacing energy-dense foods with foods containing lower energy density. As needed to promote compliance, participants underwent weeklong periods of full food provision on a 20% hypocaloric diet. Dietary advice also included recommendations for macronutrient intake to be within the recommended ranges (percentages of total energy: carbohydrate 45–65%; fat 20–35%; and protein 10–35%) (10).

EX Intervention

The EX intervention was designed to increase TEE by $\sim$20% by using EX without

changing energy intake. Weekly EX energy expenditure prescriptions were calculated after accounting for differences between gross and net EX energy expenditure, as described previously (1). The subjects monitored their progress toward the energy expenditure goals with heart rate (HR) monitors (Polar Electro Oy, Kempele, Finland), which estimate EX energy expenditure based on EX HR and subject-specific characteristics (e.g., weight, maximal oxygen uptake [VO_{2max}]). The monitors stored data for EX energy expenditure, HR, EX duration, and EX frequency, all of which were transferred to the study database each week. Specific goals for EX frequency and intensity were not provided; however, to maximize weekly energy expenditure, the participants were encouraged to perform daily EX and to mostly perform activities that required "moderate" and "hard" physical effort. The participants were advised to perform cardiovascular EX and to increase functional physical activities (e.g., active transportation). They were also advised to refrain from strength/resistance training, as it may alter glucoregulation through unique mechanisms. During the initial three to six EX sessions, and as needed to promote compliance thereafter, the participants exercised under the supervision of study personnel. Otherwise, the subjects were encouraged to EX on their own (i.e., fitness facility, home, or outdoors).

Caloric Restriction Plus EX Intervention

The CREX intervention was designed to induce weight loss through a combination of CR and EX, with each component contributing approximately half to the total energy deficit. The participants were given weekly EX energy expenditure prescriptions equal to 10% of TEE. The remainder of the energy deficit (10%) was induced by caloric restriction.

Body Weight and Composition

On 2 separate days at each study time point, fasted morning body weight was measured in duplicate while the participant was wearing a hospital gown. Fat mass and fat-free mass were measured with DXA (Lunar iDXA, software version 13.31; GE Healthcare, Madison, WI).

Energy Intake

Energy intake was quantified by using 3-day food diaries with computerized nutrient analysis (Food Processor SQL software; ESHA Research, Salem, OR).

Energy Expenditure

TEE was calculated as the average of estimates from physical activity recall (PAR) interviews and accelerometry. The PAR interview was a modified version of the Stanford 7-day PAR interview, as described elsewhere (11). Accelerometry was performed with triaxial accelerometers (RT3; StayHealthy, Monrovia, CA).

Aerobic Capacity

VO_{2max} was measured with indirect calorimetry (MedGraphics CardiO2; Medical Graphics Corporation, St. Paul, MN) during an incremental treadmill EX test to exhaustion (modified Balke treadmill protocol).

Glucoregulatory Function

Glucose tolerance and insulin action were assessed by using a 2-h frequently sampled oral glucose tolerance test (FSOGTT) (12) after an overnight fast and after 3 days of consuming ≥150 g/day carbohydrates. The oral test was used because, unlike infusion-based measures, it involves the intestine, which we proposed to be involved in the adaptations to CR. For follow-up assessments on subjects in the EX and CREX groups, tests were performed 12–24 h after EX. Venous blood samples were obtained before and at 10, 20, 30, 60, 90, and 120 min after administration of a 75-g oral glucose load for the analysis of plasma glucose (glucose oxidase method; YSI STAT Plus; YSI Life Sciences, Yellow Springs, OH), and insulin and C-peptide (IMMULITE Chemiluminescence Kit; Diagnostics Products Corporation, Los Angeles, CA).

Total areas under the curve (AUCs) for all analytes were calculated based on the trapezoidal rule. Insulin sensitivity index (ISI) was calculated according to Stumvoll et al. (13), which is reproducible (14) and valid (15), and according to Matsuda and DeFronzo (16). Total insulin secretion rate (ISR) and β-cell response were estimated by using the C-peptide minimal model (17,18) and SAAM II software (version 1.2; University of Washington Digital Ventures). Insulin clearance was estimated as the ISR AUC-to-insulin AUC ratio (19).

Matched Glucose Infusion

On a separate day after the FSOGTT, a variable rate glucose infusion was performed with the goal of matching the glycemic response from the FSOGTT. As for the FSOGTT, follow-up assessments on subjects in the EX and CREX groups were performed 12–24 h after EX. Intravenous dextrose (20%) was infused into an antecubital vein, and blood samples were drawn from the contralateral arm. Blood samples (1 mL) were drawn every 5 min for quantification of plasma glucose concentrations (YSI STAT Plus) to inform decisions about the glucose infusion rates. At the same time points as described for the FSOGTT, larger blood samples were obtained for quantification of insulin and C-peptide.

Combined results from the matched glucose infusion (MGI) and FSOGTT were used to calculate incretin effects, as follows:

$$\text{Relative incretin effect}\,(\%) = 100\times(\text{AUC}_{\text{FSOGTT}} - \text{AUC}_{\text{MGI}})/\text{AUC}_{\text{FSOGTT}}$$

$$\text{Absolute incretin effect}\,(\text{AUC units}) = \text{AUC}_{\text{FSOGTT}} - \text{AUC}_{\text{MGI}}$$

Additionally, the incretin effect on insulin clearance was evaluated by comparing insulin clearance from the FSOGTT to that from the MGI.

Incretin Hormones and Dipeptidyl Peptidase-IV

A portion of the blood samples from the FSOGTT were collected directly into tubes that contained a dipeptidyl peptidase-IV (DPP-IV) inhibitor (DPP4–010; Millipore, Billerica MA) to prevent the degradation of active forms of GLP-1 and glucose-dependent insulinotropic polypeptide (GIP). Plasma was analyzed for concentrations of active human GLP-1 (7–36 and 7–37 amides) and active human GIP (1–42 amide) with ELISA (IBL International, Toronto, ON, Canada). DPP-IV was measured in fasting plasma by using ELISA (R&D Systems, Minneapolis, MN).

Statistical Analyses

As planned a priori, the primary analyses were performed on a per-protocol basis, and excluded subjects who did not complete the study and those who did not lose weight. Baseline characteristics among groups were compared with Fisher exact tests and ANOVAs. Outcomes were compared by using ANCOVAs, in which the study group was the independent variable; change in the

outcome (i.e., final value minus baseline value) was the dependent variable, and the baseline value was a covariate. Between-group post hoc comparisons were performed using the protected *F* test principle and least significant difference tests. Baseline-adjusted least squares means were used to evaluate the significance of within-group changes. Associations were evaluated with Pearson correlations. Intention-to-treat (ITT) analyses (including data from all 69 subjects who underwent baseline testing and were randomized) were also performed. Missing data were handled by using the last observation carried forward. The analysis of outcome data included the magnitude of weight loss as a covariate. Because the ITT approach resulted in differences among groups for weight loss, which is problematic when evaluating the effects of matched weight losses, it was considered a supplementary analysis. All statistical tests were two tailed, and significance was accepted at $P \leq 0.05$. Data are presented as the arithmetic mean ± SE, unless otherwise noted. Analyses were performed using SAS for Windows (version 9.3; SAS Institute Inc., Cary, NC).

RESULTS

Participants

Among 525 individuals who inquired about study participation, 63 were not interested after learning more about the study, and 393 were screened out, with the single most common reason being BMI ≥30 kg/m^2. The remaining 69 participants were enrolled, underwent baseline testing, and were randomized (Supplementary Fig. 1, consort diagram). Twelve participants discontinued participation before providing follow-up data, and 5 participants were noncompliant (weight loss <1%; range −0.5% to 1.3%). Therefore, analyses for the present report were based on 52 participants. By design, the participants were at increased risk of diabetes by virtue of being middle to older aged (mean age 57 ± 1 years), overweight (mean BMI 27.7 ± 0.2 kg/m^2), and physically inactive (VO_{2max} values in the 10th to 15th percentile for age and sex) (20) (Table 1). Accordingly, based on fasting and 2-h plasma glucose and hemoglobin A_{1c} levels (21), 54% of the participants (*n* = 28) had prediabetes at baseline. Furthermore, although subjects with diagnosed diabetes and those with fasting blood glucose levels of ≥126 mg/dL were excluded during screening, three participants (6%) had 2-h glucose tolerance test glucose values that met the criteria for diabetes; according to clinical standards, these cases were considered "provisional diabetes" because a formal diagnosis of diabetes would have required a second test to confirm the initial results (21) (Table 1).

Table 1—Baseline characteristics of study participants

	CR group	CREX group	EX group	Among-group *P**
Participants, *n*	17	19	16	
Female sex	13 (76)	15 (79)	11 (69)	0.85
Age, years	57 ± 1	57 ± 1	56 ± 1	0.86
Race				0.26
Caucasian	16 (94)	14 (74)	12 (75)	
African American	0 (0)	4 (21)	3 (19)	
Other or not specified	1 (6)	1 (5)	1 (6)	
Body weight, kg				
Women	73.2 ± 1.8	77.2 ± 1.7	74.2 ± 1.8	0.26
Men	92.4 ± 5.6	98.7 ± 5.6	86.3 ± 5.0	0.30
BMI, kg/m^2	27.7 ± 0.4	28.3 ± 0.4	27.0 ± 0.4	0.08
Waist circumference, cm				
Women	88.6 ± 2.6	88.7 ± 1.8	90.4 ± 2.1	0.81
Men	101.9 ± 2.0	108.0 ± 4.8	96.6 ± 3.1	0.11
Energy intake, kcal/day	2,243 ± 145	2,310 ± 137	1,909 ± 149	0.12
TEE, kcal/day	2,068 ± 88	2,092 ± 86	2,143 ± 94	0.84
Activity energy expenditure, kcal/day	425 ± 43	439 ± 43	423 ± 46	0.96
VO_{2max}, mL/kg/min				
Women	25 ± 1	22 ± 1	24 ± 1	0.22
Men	32 ± 3	28 ± 3	28 ± 3	0.60
Fasting glucose, mmol/L	5.4 ± 0.1	5.4 ± 0.1	5.2 ± 0.1	0.48
2-h glucose, mmol/L	7.3 ± 0.5	6.3 ± 0.5	7.0 ± 0.5	0.37
Hemoglobin A_{1c}				
%	5.6 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	0.69
mmol/mol	38 ± 1	39 ± 1	39 ± 1	0.69
Prediabetes prevalence	9 (53)	10 (53)	9 (56)	1.00
Provisional diabetes prevalence	2 (12)	1 (5)	0 (0)	0.51
Total cholesterol, mg/dL	202 ± 7	206 ± 9	178 ± 6	0.15
Systolic BP, mmHg	127 ± 3	127 ± 3	125 ± 3	0.79
Diastolic BP, mmHg	82 ± 2	78 ± 2	82 ± 2	0.33

Values are reported as counts (%) for categorical data and the mean ± SE for quantitative data, unless otherwise indicated. Prediabetes and provisional diabetes were determined based on clinical criteria from the American Diabetes Association (21). *Between-group *P* values for quantitative data are from ANOVAs, and those for categorical data are from Fisher exact tests.

Body Weight and Composition

Body mass decreased by ~7% in all three groups (Fig. 1), as intended by design. The time required to reach the weight loss goal was shorter in the CREX group (13 ± 2 weeks) than in the CR group (19 ± 2 weeks, *P* = 0.02) and EX group (20 ± 2 weeks, *P* = 0.007). There were nonsignificant tendencies for greater fat mass reductions and better preservation of fat-free mass in the EX and CREX groups (Fig. 1). During the 2-week weight stability period prior to follow-up testing, body mass did not change (CR group −0.1 ± 0.2 kg; CREX group −0.2 ± 0.2 kg; EX group −0.2 ± 0.2 kg; all $P \geq 0.28$).

The ITT analyses revealed smaller reductions in body mass in the CR group (−4.8 ± 0.7%) and EX group (−4.6 ± 0.7%) compared with the CREX group (−7.2 ± 0.8%). Likewise, the reductions in fat mass were significantly less in the CR and EX groups (Supplementary Fig. 3).

EX Training Volume and Mode

EX energy expenditure in the CREX group was 217 ± 23 kcal/day, which is equivalent to 10% of baseline TEE. The EX energy expenditure in the EX group was 412 ± 26 kcal/day (equivalent to 22% of baseline TEE) and greater than

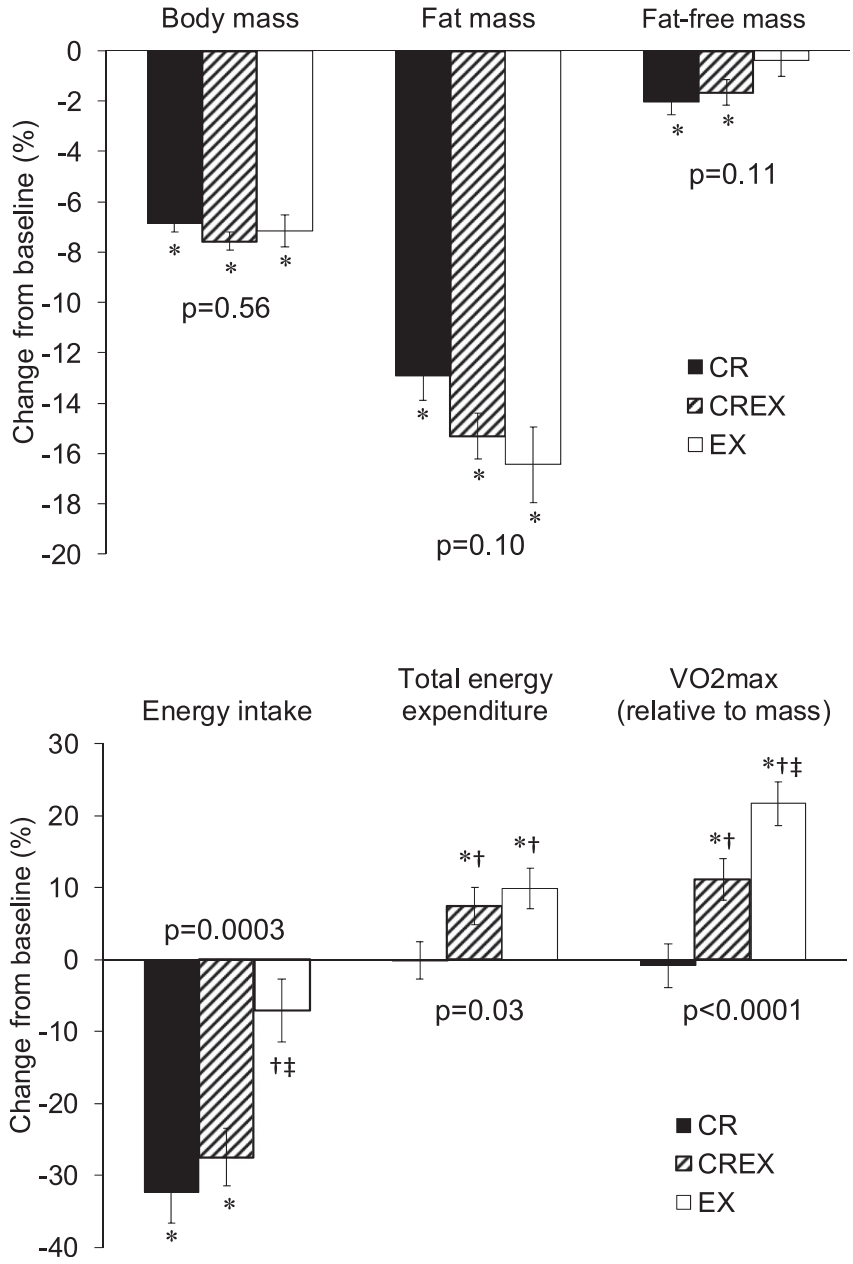

Figure 1—Body weight and composition changes (top panel) and measures of intervention compliance (bottom panel). Values are reported as the least squares mean ± SE from ANCOVAs in which the change in the outcome was the dependent variable, study group was the independent variable, and baseline values were the covariate. *P* values reflect the significance of the overall ANCOVA. When overall ANCOVAs were significant at $P \leq 0.05$, post hoc paired comparisons were performed according to the principle of protected *F* tests and least significant difference tests. *$P \leq 0.05$ vs. zero (significance of within-group change). †$P \leq 0.05$ vs. CR group. ‡$P \leq 0.05$ vs. CREX group.

that in the CREX group ($P < 0.0001$). EX duration was 4.4 ± 0.5 h/week in the CREX group and 7.4 ± 0.5 h/week in the EX group ($P = 0.0002$). EX frequency was 6 ± 1 sessions/week in the CREX group and 8 ± 1 sessions/week (i.e., >1 session/day) in the EX group ($P = 0.08$ vs. CREX). EX HR did not differ between the CREX and EX groups with respective EX HRs of 74 ± 1% and 77 ± 1% of measured maximal HR ($P = 0.17$). Brisk walking was the most common mode of EX; however, other commonly used modes included cycling, elliptical machine EX, stair climbing, and running.

Energy Intake

Energy intake in the CR and CREX groups decreased significantly, while the energy intake in the EX group was not different from baseline (Fig. 1). The reductions in energy intake in the CR and CREX groups were attributed to respective reductions in all macronutrient intakes including fat (−39 ± 7% and −27 ± 6%, both $P \leq 0.0001$), carbohydrate (−29 ± 5% and −29 ± 5%, both $P \leq 0.0001$), and protein (−22 ± 5% and −17 ± 5%, both $P \leq 0.001$). Based on the ITT analysis, which included data from participants who dropped out and those who were noncompliant, the magnitude of the decrease in energy intake in the CR group was less than that from the per protocol analysis, as expected (Supplementary Fig. 3).

Energy Expenditure

TEE increased from baseline in the EX and CREX groups and remained unchanged in the CR group (Fig. 1). As expected, due to reductions in body mass during the intervention, the increases in TEE were less than the increases in EX energy expenditure. Based on physical activity recall data, there were no changes in sedentary and light physical activity within any study group (all $P > 0.19$), and there were no differences among groups ($P = 0.96$). Compared with the per protocol analyses, the ITT analyses revealed smaller increases in energy expenditure in the CREX and EX groups (Supplementary Fig. 3).

Aerobic Capacity

Changes in VO_{2max} during the intervention corresponded with the EX dose (Fig. 1), with the CR group having no change (0 ± 1 mL/kg/min, $P = 0.79$), the CREX group having a modest increase (3 ± 1 mL/kg/min, $P = 0.0009$), and the EX group having the largest increase (5 ± 1 mL/kg/min, $P < 0.0001$). When analyzed using the ITT approach (including dropouts and noncompliant subjects), the improvements in Vo_{2max} in the CREX and EX groups were smaller than those based on the per protocol analysis (Supplementary Fig. 3).

Glucoregulatory Responses During the FSOGTT

ISI increased in all groups; however, the increase in the CREX group was twofold greater than those in the CR and EX groups (Table 2). The glucose AUC decreased in the CREX group but not in the CR or EX groups; however, these changes did not differ among the groups ($P = 0.12$) (Table 2). Among the

Table 2—FSOGTT responses

	CR	CREX	EX	Among-group *P*
ISI (Stumvoll et al. [13]), μU/kg/pM				
Baseline	0.072 ± 0.006	0.076 ± 0.009	0.085 ± 0.005	0.43
Final	0.083 ± 0.006	0.098 ± 0.005	0.093 ± 0.007	
Adjusted change	0.009 ± 0.004	0.021 ± 0.004*	0.010 ± 0.004	0.04
Within-group *P*	0.02	<0.0001	0.01	
ISI (Matsuda and DeFronzo [16] index)				
Baseline	4.4 ± 0.7	5.5 ± 0.9	5.5 ± 0.6	0.52
Final	5.3 ± 0.6	8.2 ± 1.2	6.3 ± 0.8	
Adjusted change	0.8 ± 0.6	2.9 ± 0.6*	0.7 ± 0.7	0.03
Within-group *P*	0.23	<0.0001	0.27	
Glucose AUC, mmol/L·min				
Baseline	986 ± 46	910 ± 55	897 ± 44	0.40
Final	981 ± 49	846 ± 36	921 ± 53	
Adjusted change	14 ± 34	−71 ± 32	12 ± 35	0.12
Within-group *P*	0.68	0.03	0.74	
Insulin AUC, $\times 10^3$ pmol/L·min				
Baseline	56.8 ± 6.7	56.8 ± 9.5	44.1 ± 5.8	0.43
Final	43.5 ± 3.5	43.3 ± 8.3	36.4 ± 4.8	
Adjusted change	−12.0 ± 3.4	−12.2 ± 3.2	−10.6 ± 3.6	0.94
Within-group *P*	0.001	0.0004	0.005	
C-peptide AUC, nmol/L·min				
Baseline	1,184 ± 69	1,113 ± 101	1,052 ± 80	0.58
Final	1,041 ± 64	957 ± 103	958 ± 80	
Adjusted change	−128 ± 54	−156 ± 51	−108 ± 56	0.82
Within-group *P*	0.02	0.004	0.06	
ISR AUC, $\times 10^3$ pmol				
Baseline	29.2 ± 1.8	26.9 ± 2.5	25.9 ± 2.0	0.57
Final	25.9 ± 1.7	22.7 ± 2.4	23.3 ± 2.2	
Adjusted change	−2.8 ± 1.4	−4.3 ± 1.4	−2.9 ± 1.5	0.69
Within-group *P*	0.06	0.002	0.05	
Φ, 10^9 min^{-1}				
Baseline	34.5 ± 2.1	32.9 ± 2.8	34.5 ± 2.8	0.89
Final	31.3 ± 2.2	29.0 ± 3.1	30.3 ± 2.2	
Adjusted change	−2.9 ± 2.2	−4.4 ± 2.1	−3.9 ± 2.3	0.89
Within-group *P*	0.20	0.04	0.09	
Insulin clearance index (ISR:insulin AUC ratio)				
Baseline	0.58 ± 0.04	0.57 ± 0.04	0.66 ± 0.05	0.26
Final	0.62 ± 0.03	0.64 ± 0.05	0.70 ± 0.05	
Adjusted change	0.04 ± 0.03	0.07 ± 0.03	0.05 ± 0.03	0.66
Within-group *P*	0.20	0.01	0.09	
GLP-1 AUC, pmol/L·min				
Baseline	1,357 ± 124	1,582 ± 139	1,273 ± 104	0.20
Final	1,169 ± 100	1,636 ± 157	1,407 ± 143	
Adjusted change	−199 ± 95†	86 ± 91	106 ± 99	0.05
Within-group *P*	0.04	0.35	0.29	
GIP AUC, pmol/L·min				
Baseline	4,299 ± 350	4,050 ± 398	3,445 ± 433	0.32
Final	3,898 ± 295	3,654 ± 371	3,460 ± 353	
Adjusted change	−292 ± 216	−364 ± 203	−139 ± 224	0.76
Within-group *P*	0.18	0.08	0.54	
DPP-IV (fasting), ng/mL				
Baseline	402 ± 15	371 ± 24	368 ± 24	0.48
Final	373 ± 14	375 ± 27	348 ± 25	
Adjusted change	−22.9 ± 17.3	2.0 ± 16.2	−23.2 ± 17.7	0.47
Within-group *P*	0.19	0.90	0.20	

Values are reported as arithmetic means ± SE, except for adjusted change values, which are least squares means ± SE that have been adjusted for differences in baseline values among groups. Φ, pancreatic β-cell sensitivity to glucose according to the C-peptide minimal model analysis (17,18). Insulin clearance index was calculated as the ratio of total ISR AUC to insulin AUC (19). Among-group *P*, significance of the among-group differences in change values after adjustment for baseline values using ANCOVA. *$P \leq 0.05$ vs. CR and EX groups; †$P \leq 0.05$ vs. CREX and EX groups.

participants with prediabetes at baseline (Table 1), 33% (n = 3) in the CR group, 30% in the CREX group, and 11% in the EX group became normoglycemic at the follow-up assessment; these conversion rates did not differ among groups (P = 0.46). FSOGTT insulin and C-peptide AUCs decreased to a similar extent in all study groups (Table 2). These changes were accompanied by reductions in ISR AUC (−12%, P = 0.0002). Changes in insulin clearance did not differ among groups (Table 2); however, with all groups, the combined insulin clearance increased by 9% (P = 0.002).

Based on the ITT analyses, including data from dropouts and noncompliant participants, the improvements in insulin sensitivity (the method of Stumvoll et al. [13]) were smaller than those from the per protocol analysis (0.008 ± 0.003, 0.018 ± 0.004, and 0.005 μU/kg/mM in the CR, CREX, and EX groups, respectively), and the test for differences among groups became marginally nonsignificant (P = 0.054). Results from the ISI of Matsuda and DeFronzo (16) were similarly affected, with the test for differences among groups becoming marginally nonsignificant (P = 0.08). The statistical significance of results for glucose, insulin, and C-peptide AUCs and ISR were not different between the ITT and per protocol analyses. However, the decreases in insulin AUC and ISR were smaller in the ITT analysis (insulin AUC × 10^3 pmol/L·min: CR −10.4 ± 3.3, CREX −12.2 ± 3.7, EX −5.2 ± 3.2; ISR AUC × 10^3 pmol: CR −2.5 ± 1.2, CREX −3.5 ± 1.3, EX −1.7 ± 1.1).

Incretin Hormone Responses to the FSOGTT

Plasma GLP-1 response (AUC) during the FSOGTT decreased by 15% in the CR group and remained unchanged in the CREX and EX groups (Table 2). Changes in GIP AUC did not differ among groups (P = 0.76); however, there was a marginally significant 7% decrease (P = 0.058) with all groups combined. DPP-IV concentrations did not change in any of the groups (Table 2). The statistical significance of the incretin hormone results from the ITT analyses did not differ from the results of the per protocol analyses described above. However, the magnitude of the decrease in GLP-1 AUC in response to CR was 23% smaller (−199 ± 95 vs. −153 ± 73 pmol/L·min, both P = 0.04).

Glucoregulatory Responses to the Matched Glucose Infusion

Plasma glucose concentrations from the FSOGTT and MGI were well matched (Supplementary Fig. 2). There were no differences among groups in terms of changes in MGI insulin, C-peptide, or insulin secretions rates (Supplementary Table 1). However, with all groups combined, significant decreases were observed for MGI insulin AUC (−25%, P = 0.002) and C-peptide AUC (−17%, P = 0.0003). These changes were accompanied by a 17% reduction in ISR AUC (P = 0.002). Insulin clearance during the MGI exhibited a 14% increase (P = 0.001) for all groups combined, with no differences among groups.

Incretin Effects

As expected, ISRs and postprandial insulin and C-peptide concentrations were greater after oral glucose ingestion than during the glycemia-matched glucose infusion, indicating the presence of incretin effects (Table 3 and Supplementary Fig. 2). None of the interventions altered the absolute or relative incretin effects on insulin secretion (Table 3). Likewise, the relative incretin effects on insulin AUC and C-peptide AUC did not change. Although no among-group differences were observed, absolute incretin effects on insulin and C-peptide decreased with weight loss when all groups were combined (−6.6 ± 2.4 × 10^3 pmol/L·min [P = 0.009] and −919 ± 42 pmol/L·min [P = 0.002], respectively). Insulin clearance was lower after oral glucose infusion than during the matched glucose infusion, indicating an incretin effect to suppress insulin clearance; the magnitude of this incretin effect did not change during any of the interventions (Table 3). The use of ITT analyses did not alter the significance of the incretin effect results (i.e., neither the ITT or per protocol analysis yielded any significant differences among groups).

CONCLUSIONS

Weight loss involving CR and EX improves glucoregulation and reduces diabetes risk in overweight and obese subjects (2,3); however, the independent contributions of CR and EX are poorly understood. Results from the current study demonstrate that CR and EX together improve insulin sensitivity twofold more than does the same amount of weight loss induced by CR alone or EX alone. Furthermore, to ensure similar weight losses in the three groups, the CREX group underwent less CR and less EX training than those used in the CR and EX groups, respectively, and, despite this, they had much larger improvements. The finding of additive effects indicates that some of the adaptations to CR are mechanistically distinct from those caused by EX-induced weight loss. Thus, although a combination of CR and EX is often recommended for maximizing weight loss because both contribute to a negative energy balance, findings from the current study provide an additional rationale for encouraging both CR and EX, because together they provide a greater improvement in insulin sensitivity than either alone.

A secondary objective of the current study was to gain insights about distinct mechanisms by which CR improves glucoregulation. Postprandial GLP-1 concentrations decreased in response to CR, but not in response to matched weight loss from EX. Others have also reported that CR decreases postprandial GLP-1 levels (22,23) (as long as the postprandial response at baseline is not blunted [24]), and that EX training does not affect GLP-1 concentrations (25,26). Because GLP-1 influences glycemic control through actions on the pancreas, skeletal muscle, and adipose tissue (7–9), the finding of CR-induced reductions in GLP-1 in the current study raises the possibility that some of the beneficial effects of CR on glucoregulation may involve GLP-1. In contrast to the findings for GLP-1, changes in postprandial GIP concentrations did not differ among groups. However, with all groups combined, a marginal decrease in GIP occurred (P = 0.058), suggesting that weight loss, per se, mediates this effect. Others have reported similar GIP responses to weight loss (24,27,28) and that EX training without weight loss does not alter GIP (25,28).

It is plausible that the observed reductions in GLP-1 and GIP levels might have resulted from reduced intestinal secretion. Because we measured the bioactive forms of these hormones, an alternate explanation would be enhanced degradation to the inactive

Table 3—Absolute and relative incretin effects

	CR	CREX	EX	Among-group *P*
Incretin effect on ISR total AUC, %				
Baseline	53.2 ± 3.0	56.6 ± 4.1	43.2 ± 8.2	
Final	53.2 ± 4.2	59.5 ± 2.9	41.4 ± 7.5	0.27
Adjusted change	0.5 ± 3.6	5.6 ± 3.5	2.7 ± 3.6	
Within-group *P*	0.89	0.12	0.47	0.60
Incretin effect on ISR AUC, $\times 10^3$ pmol				
Baseline	16.2 ± 1.6	14.1 ± 1.5	12.2 ± 1.3	0.19
Final	14.0 ± 1.4	12.9 ± 1.4	12.1 ± 1.4	
Adjusted change	−1.0 ± 1.3	−1.2 ± 1.2	−1.1 ± 1.3	1.00
Within-group *P*	0.44	0.34	0.39	
Incretin effect on insulin AUC, %				
Baseline	63.2 ± 3.6	68.2 ± 3.0	55.1 ± 5.5	0.09
Final	63.9 ± 4.4	69.1 ± 3.5	63.6 ± 4.5	
Adjusted change	1.4 ± 4.1	5.4 ± 4.1	3.0 ± 4.2	0.79
Within-group *P*	0.73	0.19	0.48	
Incretin effect on insulin AUC, $\times 10^3$ pmol/L·min				
Baseline	38.4 ± 5.7	36.8 ± 7.5	25.5 ± 4.2	0.27
Final	28.4 ± 2.9	30.3 ± 7.3	22.3 ± 2.8	
Adjusted change	−8.1 ± 3.5	−5.2 ± 3.4	−6.5 ± 3.5	0.84
Within-group *P*	0.03	0.13	0.07	
Incretin effect on C-peptide AUC, %				
Baseline	82.7 ± 1.0	84.0 ± 1.3	80.4 ± 1.5	0.14
Final	82.7 ± 1.2	84.5 ± 0.9	83.2 ± 1.4	
Adjusted change	0.2 ± 1.1	1.6 ± 1.1	1.4 ± 1.1	0.64
Within-group *P*	0.86	0.16	0.22	
Incretin effect on C-peptide total AUC, nmol/L·min				
Baseline	1,001 ± 65	912 ± 84	847 ± 65	0.33
Final	882 ± 55	790 ± 95	795 ± 65	
Adjusted change	−100 ± 52	−124 ± 50	−69 ± 52	0.75
Within-group *P*	0.06	0.02	0.19	
Incretin effect on insulin clearance, %				
Baseline	−36 ± 7	−40 ± 6	−26 ± 7	0.32
Final	−36 ± 6	−38 ± 7	−38 ± 7	
Adjusted change	−2 ± 7	−4 ± 7	−5 ± 7	0.96
Within-group *P*	0.77	0.59	0.49	
Incretin effect on insulin clearance, ISR:insulin AUC ratio				
Baseline	−0.19 ± 0.04	−0.21 ± 0.03	−0.15 ± 0.04	0.45
Final	−0.22 ± 0.04	−0.24 ± 0.05	−0.26 ± 0.06	
Adjusted change	−0.04 ± 0.05	−0.05 ± 0.05	−0.08 ± 0.05	0.80
Within-group *P*	0.47	0.32	0.11	

Values are reported as the arithmetic mean ± SE, except for adjusted change values, which are reported as the least squares mean ± SE that have been adjusted for differences in baseline values among groups. Among-group *P*, significance of the among-group differences in change values after adjustment for baseline values using ANCOVA. Incretin effect data are missing for one subject in the CR group and two subjects in the CREX group because of missing matched glucose infusion data (refused test *n* = 2, technical problems during test *n* = 1).

forms by DPP-IV, which occurs within minutes after secretion (29,30). However, because DPP-IV concentrations did not change, this scenario does not seem likely.

Despite the reductions in postprandial incretin hormone levels, none of the weight loss interventions affected the incretin effects on insulin secretion, clearance, or concentrations, suggesting that the net effect of the gut on insulin metabolism was not altered. This dissociation of incretin hormone concentrations from incretin effects suggests that the β-cells of the pancreas became more sensitive to GLP-1 (CR group only) and GIP (all groups). Other studies on the effect of weight change on incretin effects are mixed, with one showing that CR had no effect (31), another showing that short-term weight gain reduced the incretin effect from 72% to 43% (32), and others reporting twofold to fivefold increases from weight loss after gastric bypass (31,33,34).

Data from the current study indicate that CR and EX have additive effects on insulin sensitivity when weight loss is matched. However, this finding might understate the benefits of combined CR and EX because together, they would likely lead to greater weight loss success. Evidence from the current study supports this notion. First, the ITT analysis of weight changes (including all subjects, regardless of withdrawals and poor compliance) revealed ~50% greater weight loss in the CREX group than in the other groups. Furthermore, despite the fact that the interventions were designed for similar rates of weight loss, the CREX group attained the weight loss goal in one-third less time than the other groups. Additionally, 95% of the subjects randomized to CREX eventually achieved the

target weight, while only two-thirds of the CR and EX group subjects reached the weight loss goal. These findings related to weight loss efficacy, in addition to the weight loss–independent benefits of CR and EX, underscore the importance of using both a low-calorie diet and EX for minimizing diabetes risk.

The current study has limitations. First, the study was not powered to detect improvements in clinical status (i.e., prediabetes vs. normoglycemia), especially after eliminating participants who had normoglycemia at baseline. Future studies with more participants and that target individuals with prediabetes are warranted. Another limitation is that we enrolled only overweight individuals because obese men and women would be at an increased risk of orthopedic problems during vigorous EX. Therefore, it is not clear whether these findings can be generalized to obese individuals.

Another limitation is that the primary statistical analyses were performed using a "per protocol" analysis, which excluded data from participants who withdrew from the study and those who did not adhere to the interventions. ITT analyses including data from all randomized participants are the standard for clinical trials and are especially important for evaluating the effectiveness of clinical treatments in a real-world setting. However, the current study was not an effectiveness trial; rather, it was designed to compare the efficacy of the interventions when fully adhered to. Furthermore, ITT analyses were not ideal for the current study because the CR and EX groups had lower adherence and more withdrawals, and consequently less weight loss when using an ITT analysis (Supplementary Fig. 3); this difference in weight loss among groups precludes the evaluation of the weight loss–independent effects of CR, CREX, and EX, and might lead to the invalid conclusion that the differences in glucoregulation among groups are attributable to differences in weight loss. Nonetheless, ITT analysis was performed as a secondary analysis, and the results were adjusted for the differences in weight loss among groups (this adjusts the results to reflect an average weight loss of −5.4% based on all subjects, regardless of compliance). While the results from this analysis largely support those from the per protocol analysis, most of the improvements in outcomes were attenuated, and the differences among groups for insulin sensitivity became marginally nonsignificant (P = 0.054). A limitation in using per protocol analyses is that it might cause a selection bias. That is, some of the benefits of random allocation may be lost, and the results may be attributable to unknown characteristics that differed among groups. Therefore, the results from the current study should be interpreted with this possibility in mind.

In conclusion, data from the current study indicate that a combination of CR and EX results in greater improvements in glucoregulation than matched weight loss induced by CR or EX alone. This finding of additive benefits suggests that both CR-induced and EX-induced weight loss provide mechanistically distinct adaptations that may not be directly attributable to weight loss. Although preliminary, the CR-specific adaptation may involve alterations in postprandial GLP-1 secretion and actions. From a clinical perspective, these findings underscore the importance of recommending both a healthy low-calorie diet and EX for minimizing the risk of type 2 diabetes.

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