



## Metabolic parameters in smokers undergoing smoking reduction

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### ABSTRACT

**Introduction:** Few human studies have explored the mechanisms of smoking-induced insulin resistance. **Aims:** To prospectively examine the metabolic changes of smoking reduction.

**Methods:** Cigarette smokers (n = 22; ½–2 packs per day) were enrolled in a smoking reduction program (counseling plus bupropion × 8 weeks; Phase I) followed by monitoring only (no counseling or bupropion × 16 weeks; Phase II). We serially measured exhaled carbon monoxide (CO) and urine nicotine metabolites; fat distribution, and metabolic parameters by hyperinsulinemic clamps including hepatic glucose output (HGO) and indirect calorimetry, adjusted for total caloric intake and expenditure.

**Results:** CO and nicotine metabolite levels fell with smoking reduction during Phase I (all p < 0.05), without any further changes through Phase II. Central-to-peripheral fat ratio increased during Phase I, but then fell during Phase II (all p < 0.05). Over 24 weeks, basal HGO fell (p = 0.02); and falling CO and nicotine metabolite levels correlated inversely with changes in glucose oxidation, and directly with changes in weight (all p < 0.05).

**Conclusions:** Smoking reduction produced a transient worsening of central fat redistribution followed by a more significant improvement; along with other net beneficial metabolic effects.

### Introduction

While the prevalence of smoking among US adults has declined from 42.4% in 1965 to 14% in 2017 [1], cigarettes still are recognized as the single largest preventable cause of death [2]. Smoking is a major cardiovascular risk factor [3–5], but dynamic measures of glucose uptake have also shown that smokers are more insulin resistant compared to non-smokers [6–10], with the degree of insulin resistance correlated with tobacco consumption [7] and markers of nicotine use [11,12]. In population-based studies, cigarette smoking was associated with an increased incidence of diabetes mellitus [13,14]; some 12% of diabetes in the U.S. may be attributable to smoking [15]. Intervention studies have shown that smoking cessation was accompanied by improvements in insulin sensitivity [6,16,17] with as little as 6–8 weeks of cessation [6,17] or withdrawal of nicotine replacements [6], although in a recent intervention study, fasting insulin sensitivity deteriorated along with

increased  $\beta$ -cell secretion in response to glucose [18]. In contrast, population-based studies have shown that recent smoking cessation may be associated with an increased rate of diabetes, which may or may not be explained by weight changes [19,20], but coinciding with higher waist circumference, BMI and insulin resistance [21]. Kim et al. [22] found that the longer the smoking cessation period, the more the insulin resistance tended to decrease in asymptomatic Korean male ex-smokers, while in a study of Japanese men, hemoglobin A1c, but not fasting plasma glucose, decreased linearly with increase in years after smoking cessation [23]. Smoking is also dose-dependently associated with increased central and visceral adiposity, independent of overall weight changes, in some [24,25], but not all [26] cross-sectional studies. Elevated hepatic glucose output (HGO), another feature of insulin resistance, has not been extensively studied in relation to smoking cessation; one short-term cessation study found a non-significant improvement [16], while another did not [27].

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We prospectively examined changes in detailed metabolic assessments (body composition, fat distribution, and insulin resistance using hyperinsulinemic euglycemic clamps coupled with measures of HGO and substrate utilization) in smokers who stopped or reduced smoking, and who might then return to smoking; over a 6-month period.

## Patients and Methods

We conducted a prospective, single-arm intervention study and examined metabolic parameters measured before and after an intensive 8-week smoking reduction program, and again after a further 16-week period of smoking reduction maintenance without counseling (and thus possible smoking resumption). This unique study design allows for a comparison of physiological changes between the smoking and reduced-smoking states, transitioning in both directions, without violating ethical requirements. The Institutional Review Board of Charles R. Drew University of Medicine and Science approved the protocol (Clinicaltrials.gov #NCT00877513) in accordance with U.S. Federal Policy for the Protection of Human Subjects. This institution serves a low-income, racial and ethnic minority population with a disproportionate prevalence of obesity and insulin resistance.

### Subjects

Chronic ( $\geq 3$  years) cigarette smokers, age 25–70, smoking between  $\frac{1}{2}$  to 2 packs per day were recruited through direct advertising within the local communities served by our institution, as well as print, radio and online advertisements across Southern California. All subjects provided informed consent. Subjects had a body mass index (BMI) between 19 and 45 kg/m<sup>2</sup>, and no history of cardiovascular or pulmonary diseases, diabetes mellitus, uncontrolled hypertension or hyperlipidemia, unstable living conditions, seizure or depression history that would contraindicate the use of bupropion, or any other conditions that could complicate data validity or safety. Subjects actively engaged in smoking reduction efforts, with or without pharmacological aids were excluded, as were *peri*-menopausal (within 6 months), pregnant or lactating women, and any subjects concurrently using weight loss agents or any hormonal therapies (e.g., oral contraceptives, thyroid medications) that were not at stable dosages; any such concurrent medications remained constant throughout the study. The Simple Screening Instrument for Alcohol and Other Drugs (SSI-AOD) [28] was administered during screening to detect excessive use of alcohol or other illicit substances; anyone scoring  $\geq 4$  was thought to be less likely to quit smoking and was excluded.

### Study procedures

Qualifying subjects were characterized at baseline with respect to smoking severity, anthropometry (weight, height, BMI), and body composition (fat and lean mass) using a Hologic 4500 dual x-ray absorptiometry (DXA) scanner (Hologic, Bedford, MA). Self-reported smoking severity was corroborated using both the mean of 3 replicate measures of breath CO (in parts per million, ppm) using the Micro 4 Smokerlyzer CO monitor (Bedfont Scientific, Williamsburg, VA), calibrated according to manufacturer's recommendations; and measures of urine nicotine and nicotine metabolites measured semi-quantitatively (on a discrete scale of 0 to 14) using the NicCheck I Test Strips (measures urine nicotine, cotinine, and 3-hydroxycotinine; Mossman Associates, Inc., Millford, MA; 98.4% and 96.1% positive- and negative predictive values, respectively, as compared to gas chromatography) on a freshly voided urine sample. All subjects also underwent a non-contrast CT scan on a Siemens Sensations MultiSlice 16-Channel CT scanner (Siemens USA, Washington DC) with image analysis performed on an Infinitt Diagnostics PACS workstation (Infinitt North America, Phillipsburg, NJ) by dedicated radiologists to measure hepatic, abdominal, and thigh fat using a single slice (3 mm section) image

landmarked at, respectively, the xiphoid process and ribs (liver and spleen), the iliac crest and L3/L4 vertebrae (abdominal), and the proximal 1/3 of the right thigh from the hip to the knee joint (thigh). Hepatic fat was determined as the hepatic attenuation index, defined as Hounsfield units at the spleen subtracted from those at the liver [29], quantitated at a symmetric 1-cm diameter region of interest chosen at the center of the spleen and right liver lobe away from vascular structures. Abdominal visceral, abdominal subcutaneous and thigh fat areas were determined using hand-drawn regions of interest on each respective image, relative to total area.

All qualifying subjects underwent a baseline 4-hour hyperinsulinemic euglycemic clamp. Smoking was not permitted in the morning prior to the procedure. Intravenous access was established in both antecubital fossae of the fasting subject, followed by a 200 mg/m<sup>2</sup> IV bolus of 6,6-D<sub>2</sub>-glucose in saline (stable isotope tracer, Cambridge Isotopes, Tewksbury, MA) and a continuous 2 mg/m<sup>2</sup>/min infusion for  $\geq 3$  h and then continuing throughout the procedure. Indirect calorimetry using a VMax Encore calorimeter (CareFusion, San Diego, CA) and a mixing chamber (canopy) was applied to the relaxed, supine, awake subject for 15 min prior to and at the end of each hour of the insulin infusion period; airflow was adjusted between 30 and 50 mL/min to maintain fractional expired CO<sub>2</sub> between 0.5 and 1.0%; rates of oxygen consumption (VO<sub>2</sub>) and CO<sub>2</sub> production (VCO<sub>2</sub>) measurements were recorded each minute. Warming pads were then applied to the sampling IV site and a 4-hour infusion of regular human insulin (Novolin R, Novo Nordisk U.S., Plainsboro, NJ) was started at rates of 5, 10, 20 and 80 mU/m<sup>2</sup>/min for 60 min each. Plasma glucose was measured at the bedside every 5 min (YSI 2300 Stat Plus Glucose Analyzer, YSI Inc., Yellow Springs, OH), and 20% dextrose enriched with 2.5% tracer was infused at varying rates to maintain glucose levels at 100  $\pm$  5 mg/dL. Throughout the procedure, additional samples were collected for free fatty acids (every hour), tracer (every 20 min), and plasma insulin (every 10 min prior to the procedure and for the final 30 min of each hour).

Maximal insulin-stimulated peripheral glucose uptake (IMGU) was calculated from the mean dextrose infusion rate in the final 30 min of the clamp procedure, with and without adjustment for the mean serum insulin level, body weight, and/or lean body mass. Plasma insulin was assayed using the Millipore Human Insulin RIA kit (EMD Millipore, Billerica, MA) following manufacturer's protocols; and HGO by gas chromatography-mass spectrometry determination of isotopic enrichment, calculated using the non-steady state equations of Steele for stable isotope [30]. Rates of glucose oxidation (GOx) and the respiratory quotient (RQ) were calculated based on mean VO<sub>2</sub> and VCO<sub>2</sub> from the final 10 min of the calorimetry periods at baseline and at the 80 mU/m<sup>2</sup>/min insulin infusion, using established equations [30]. Non-oxidative glucose uptake (NGU) was derived by subtracting GOx from IMGU. Timed urine samples for urinary nitrogen excretion were collected before and throughout the insulin infusion to correct for changes in urinary nitrogen clearance, using modified equations [31].

Subjects were specifically instructed not to alter their smoking habits prior to the start of counseling. Upon completion of the above baseline measures, subjects began Phase I, an 8-week program of weekly cognitive behavioral therapy counseling for smoking reduction, delivered by a certified addiction counselor (C.G.), and focusing on recognition, avoidance, and coping skills related to situations that increase cigarette cravings. The behavioral program was supplemented by oral bupropion hydrochloride for all subjects (Anda Pharmaceuticals, Atlanta, GA), 150 mg daily increasing to twice daily after 3 days as per FDA-approved labeling. All subjects completed a 3-day dietary recall questionnaire each week, and mean daily caloric intake was estimated using the Nutritionist Pro database (v. 4.0; Axxya Systems, Redmond, WA). Mean daily caloric expenditure was estimated using the SenseWear Pro armband (Body Media Inc., Pittsburgh, PA), worn continuously for 3–4 consecutive days each week, including weekends; and derived using the manufacturer's software (SenseWear Professional, version 6.1). Subjects completed smoking diaries daily and discussed their entries with the

counselor at each weekly visit, reviewing their progress, challenges, cravings, sensory cues and triggers that interfered with reduction efforts or abstinence, psychological and emotional barriers, and reinforcement of cognitive strategies and skills to mitigate resumption or relapse. Breath CO and urine nicotine metabolite determinations were repeated at each weekly visit, as was screening for new-onset depression to detect any adverse reactions to bupropion.

All outcome measures, including scans and all clamp measures were repeated at the end of Phase I (post-reduction, 8-weeks, see Fig. 1), prior to bupropion discontinuation. Subjects who substantially reduced their smoking ( $\leq 50\%$  of their baseline rate) continued into Phase II, a 16-week period of smoking reduction maintenance wherein visits continued only once each month (without counseling or bupropion; smoking diaries were still completed daily, but dietary recalls and armband determinations were completed monthly). Subjects who failed to substantially reduce smoking through Phase I were withdrawn after all post-reduction measures were obtained. Subjects who completed the

full study (24 weeks) then underwent a final repeat measurement of all outcome measures at study end.

#### Analyses

Only subjects who provided complete data at the end of Phase I were analyzed; missing data points were not imputed because of the exploratory nature of the study. Data from both sexes were combined. Outcome measures included smoking severity expressed as mean number of cigarettes smoked per day, mean breath CO, and urine metabolite levels; mean daily caloric intake and expenditure as important confounders; weight, BMI, and relative fat and lean mass; hepatic attenuation index and measures of abdominal (visceral and subcutaneous) and thigh fat and their ratios; baseline HGO with and without adjustment for weight and fat mass; maximal IMGU and NGU (with and without adjustment for insulin and weight or lean mass); and baseline and maximally-stimulated GOx rates (with and without adjustment for

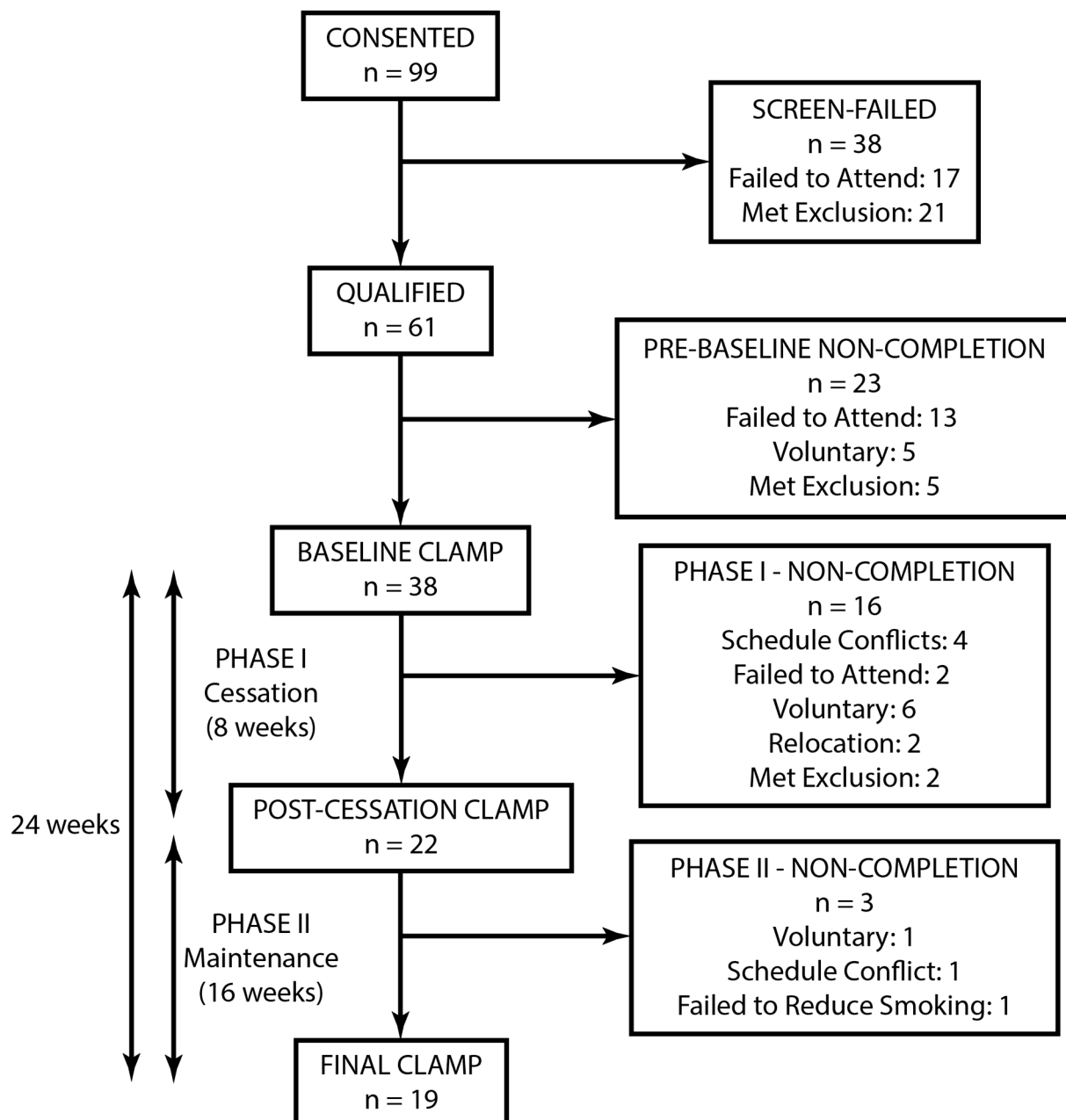


Fig. 1. Study Timeline and Flow of Subjects.

weight and lean mass) and RQ. For all outcome measures, baseline (pre-reduction) levels were compared to post-reduction levels (Phase I). For subjects completing Phase II, post-reduction levels were compared with those at study end (Phase II); the overall 24-week changes from baseline to study end were also compared. The Wilcoxon signed-rank test was used for all such pairwise comparisons; differences that achieved or approached significance ( $p \leq 0.10$ ) were further adjusted for changes in caloric intake and expenditure over the respective time periods, using the repeated measures ANCOVA. Given the exploratory nature of the study's outcomes, correction for multiple comparisons was not applied; statistical significance remained at  $p < 0.05$ . For each of the three time intervals of interest, Pearson's correlation coefficients were also determined between the changes in CO and urine metabolite levels and the corresponding changes in metabolic outcome measures, with and without adjustment for caloric intake and expenditure; statistical significance corresponded to  $r = \pm 0.42$ , but because of missing data points for some measures, a more conservative  $r = \pm 0.47$  was used for significance at  $p < 0.05$ .

## Results

### Subject characteristics

The study's overall timeline and flow of subjects are shown in Fig. 1. Out of 99 subjects screened, 61 qualified, although 23 of these subjects never provided baseline data. The remaining 38 subjects completed baseline assessments, but 16 of these subjects failed to complete Phase I. Thus, 22 subjects provided complete post-smoking reduction data and were analyzed. Table 1 shows their characteristics at screening (predominantly African-American males, mean age  $46 \pm 11$  years, smoking  $1.0 \pm 0.5$  packs per day). Three of the 22 subjects failed to continue into Phase II. All subjects tolerated the bupropion, with only minor reports of a change in taste sensation that for most subjects enhanced their efforts to reduce smoking or abstain; no subjects discontinued bupropion due to side effects.

### Changes over time

Our smoking reduction program was highly successful (Table 2); median (interquartile range) number of cigarettes per day fell from 8.8 (6.5–12.3) at the start of their program to 1.4 (0.1–3.3) by the end of Phase I, coinciding with significant reductions in both breath CO and urine metabolite levels. There were no further significant changes thereafter during Phase II, and the overall change over the full 24-weeks remained significant. Caloric intake did not change significantly, but caloric expenditure fell during Phase I. Weight, BMI, body composition,

and hepatic fat did not change significantly. However, ratios of central-to-peripheral body fat distribution increased significantly during Phase I, then fell during Phase II (despite no concurrent changes in smoking severity in Phase II), such that the overall fat distribution change over 24 weeks was not significant (Table 2). Among metabolic measures, HGO fell significantly after 24 weeks (although attenuated after adjustment for caloric intake and expenditure), but it did not change significantly solely within Phase I when most of the smoking reduction took place. We found no significant changes over time in any measures of IMGU, GOx, or NGU.

### Correlations with smoking severity

Table 3 shows statistically significant correlation coefficients between changes in the markers of smoking severity (CO and urine metabolite measures) and the metabolic measures, with and without adjustment for caloric intake and expenditure. Changes in body composition, hepatic fat and NGU failed to correlate during any phase. During Phase I, the reduction in CO correlated inversely with changes in the ratios of fat distribution, independent of caloric intake and expenditure. In Phase II, the change in CO, although not significant by itself, still correlated inversely with adjusted rates of IMGU. Over the full 24-weeks, the reduction in CO correlated directly with the change in weight-adjusted HGO, and inversely with the change in basal and stimulated rates of weight-adjusted GOx and RQ; the reduction in urine metabolites correlated directly with the change in weight, and inversely with the change in maximally-stimulated RQ and weight-adjusted rates of GOx.

## Discussion

Our study is the first to prospectively explore the changes in fat distribution and glucose metabolism that occur with smoking reduction/cessation, longer-term maintenance, and/or potential smoking resumption, using rigorous dynamic measures. Our study design is also unique, in that it uses the natural recidivism that occurs over time after a reduction/cessation program ends (thus reflecting real-life) to explore the effects of bi-directional changes in smoking severity, while avoiding ethical constraints (such as a control group being asked not to quit, or deliberately increasing cigarette use). Unfortunately, we could not directly compare those who sustained their reduction versus those who relapsed because so few subjects relapsed.

Although we failed to observe overall body weight changing significantly with reduction, this is not inconsistent with the findings of our own literature review [32] in that weight gain does not always occur with cessation. We found a worsening of central fat redistribution within Phase I, followed by a significant improvement over Phase II, such that there was no significant net difference over 24 weeks. These effects were largely independent of caloric intake and expenditure, and were inversely correlated with changes in CO. These observations suggest that the influence of smoking on body fat distribution is complex, may be biphasic, but is generally favorable over the longer-term. This is consistent with the cross-sectional observations of Lee *et al.* [25] who found that smokers who had quit within 2 years or less had greater visceral adipose than current smokers. Matsushita *et al.* [26] found a similar relationship in recent ex-smokers as compared to current smokers, with visceral and subcutaneous adipose tissue gradually declining with greater years of abstinence. Our findings may reflect this same phenomenon, but as a prospective (albeit shorter-term) observation.

HGO fell significantly over 24 weeks, but not solely within Phase I when most of the smoking reduction occurred, suggesting that this may be a more gradual process that occurs only with sustained abstinence. Hellerstein *et al.* [27] previously found no significant HGO change with smoking cessation, while Bergman *et al.* [16] found an improvement of glucose rate of appearance that just missed statistical significance. The

**Table 1**  
Subjects' Characteristics at Screening.

N	22
Age (yrs)	$46 \pm 11$
Gender (M/F)	13/9
Race/Ethnicity (Caucasian/African-American/Latino)	5/13/4
Smoking Severity (packs/day)	$0.98 \pm 0.47$
Weight (kg)	$86.6 \pm 19.6$
BMI ( $\text{kg}/\text{m}^2$ )	$30.9 \pm 8.1$
Relative Fat Mass (%)	$28.3 \pm 12.2$
Relative Lean Mass (%)	$68.8 \pm 11.5$
Systolic Blood Pressure (mm Hg)	$129 \pm 14$
Diastolic Blood Pressure (mm Hg)	$77 \pm 10$
Total Cholesterol (mg/dL)	$176 \pm 30$
Fasting Triglycerides (mg/dL) (Median, IQR)	84, 63–143
LDL-Cholesterol (mg/dL)	$104 \pm 24$
HDL-Cholesterol (mg/dL)	$49 \pm 13$
Non-HDL-Cholesterol (mg/dL)	$127 \pm 29$
Fasting Glucose (mg/dL)	$90 \pm 11$

All data represent mean  $\pm$  SD unless otherwise noted. IQR, interquartile range.

**Table 2**  
Changes in Outcome Measures Over Time.

	Pre-Cessation	Post-Cessation	Study End	P values		
				Phase I	Phase II	24 Weeks
Smoking Severity						
Mean Cigs per Day, (Median, IQR)	9.3 ± 4.2(8.8, 6.5–12.3)	2.6 ± 4.3(1.4, 0.1–3.3)	3.1 ± 4.7(1.0, 0.0–6.1)	0.00008* (0.000002*)	0.28	0.0004* (0.00009*)
Breath CO (ppm)(Median, IQR)	10.0 ± 8.7(8.9, 5.3–11.8)	5.5 ± 5.0(4.7, 1.3–8.3)	6.6 ± 8.3(3.7, 1.0–8.7)	0.002*(0.01*)	0.89	0.01*(0.02*)
Urine Metabolites (scale of 0–14) (Median, IQR)	3.9 ± 2.7(4.0, 1.8–6.0)	2.4 ± 2.2(2.0, 0.8–3.0)	2.6 ± 2.6(1.0, 0.0–5.0)	0.03*(0.06)	0.95	0.16
Caloric Intake and Expenditure						
Mean Calorie Intake (kcal/day)	1986 ± 747	1897 ± 816	1878 ± 739	0.70	0.39	0.43
Mean Calorie Expenditure (kcal/day)	2646 ± 740	2240 ± 664	2288 ± 872	0.025*	0.89	0.10
Anthropometry / Body Composition						
Weight (kg)	86.6 ± 19.6	85.7 ± 18.9	85.7 ± 17.1	0.12	0.78	0.67
BMI (kg/m <sup>2</sup> )	30.9 ± 8.1	30.7 ± 7.9	31.1 ± 7.8	0.30	1.0	0.76
FM (%)	28.3 ± 12.2	28.0 ± 11.7	28.9 ± 11.9	0.13	0.64	0.32
LM (%)	68.8 ± 11.5	69.1 ± 11.0	68.2 ± 11.2	0.10(0.28)	0.73	0.29
CT Measures: Hepatic, Abdominal (Abd) Subcutaneous (Sc), Visceral (Visc), and Thigh Fat Areas						
Total Abd Fat (% of Area)	50.7 ± 18.3	53.6 ± 18.1	53.7 ± 18.6	0.11	1.0	0.09 (0.11)
Abd Sc Fat (% of Area)	39.8 ± 15.2	41.4 ± 15.2	42.2 ± 15.6	0.73	0.92	0.46
Abd Visc Fat (% of Area)	10.8 ± 5.6	12.2 ± 6.2	11.6 ± 5.4	0.11	0.87	0.30
Thigh Fat (% of Area)	39.4 ± 18.8	37.0 ± 19.8	42.1 ± 18.3	0.34	0.03* (0.03*)	0.37
Total Abd Fat %-to-Thigh Fat % Ratio	1.5 ± 0.6	1.7 ± 0.5	1.4 ± 0.4	0.01*(0.06)	0.01* (0.04*)	0.64
Abd Sc Fat %-to-Thigh Fat % Ratio	1.1 ± 0.4	1.3 ± 0.4	1.1 ± 0.3	0.06(0.15)	0.01* (0.02*)	0.26
Abd Visc Fat %-to-Thigh Fat % Ratio	0.33 ± 0.26	0.40 ± 0.29	0.29 ± 0.15	0.04*(0.02*)	0.04* (0.16)	0.92
Hepatic Glucose Output (HGO)						
HGO/Weight (mg/kg/min)	1.90 ± 0.42	1.78 ± 0.39	1.67 ± 0.43	0.31	0.22	0.04*(0.13)
Peripheral Insulin-Mediated Glucose Uptake (IMGU)						
IMGU/LM (Insulin-Adjusted) (mg/kg/min/ (mU/L))	0.098 ± 0.044	0.100 ± 0.042	0.103 ± 0.048	0.56	0.24	0.43
Glucose Oxidation Rate (GOx)						
Basal GOx/Weight (mg/kg/min)	1.17 ± 0.48	1.30 ± 0.54	1.23 ± 0.70	0.38	0.11	0.81
Non-Oxidative Glucose Uptake Rate (NGU)						
Max NGU/LM (Insulin-Adjusted) (mg/kg/ min/(mU/L))	0.0721 ± 0.0376	0.0725 ± 0.0318	0.0768 ± 0.0372	0.49	0.16	0.61

All data represent mean ± SD unless otherwise noted. P values in parentheses are those adjusted for respective values of calorie intake and calorie expenditure. \* indicates p value is statistically significant ( $p < 0.05$ ). Phase I represents the change from pre-cessation (baseline) to post-cessation after 8 weeks; Phase II represents the change from post-cessation to study end after another 16 weeks; Overall represents the change from pre-cessation to study end after the full 24-week study. Abd, abdominal; BMI, body mass index; Cigs, cigarettes; CO, carbon monoxide; FM, fat mass; GOx, glucose oxidation rate; HGO, hepatic glucose output; HU, Hounsfield units; IMGU, insulin-mediated glucose uptake; IQR, interquartile range; LM, lean mass; Max, maximum; NGU, non-oxidative glucose uptake; RQ, respiratory quotient; Sc, subcutaneous; Visc, visceral.

fact that their cessation programs lasted only 1 and 2 weeks, respectively, is consistent with our finding that a longer follow-up may be needed to detect a more robust change in HGO. We also found that weight-adjusted HGO correlated directly with changes in CO, consistent with the notion that increased hepatic insulin sensitivity may be associated with reduced CO exposure.

Our lack of a significant change in peripheral IMGU contrasts with previous reports showing improved insulin sensitivity with cessation [6,16,17]. However, the subjects of Assali *et al.* [6] and Eliasson *et al.* [17] were heavier smokers than ours, and those of Bergman *et al.* [16] were exposed to an acute, high dose of nicotine (8 cigarettes within 4 h) just before their baseline test; the free-living smoking habits of our subjects at baseline were substantially less intense (only  $9 \pm 4$  cigarettes per day). Although our IMGU measures did not change significantly, they trended upwards over time. Changes in CO levels correlated inversely with changes in IMGU, but only during Phase II, which suggests that the influence of falling CO on improvements in IMGU may also be a gradual process, manifesting over several months.

With sustained reduction over 24 weeks, the decreases in CO and urine metabolites correlated with increases in basal and maximally-stimulated measures of RQ and GOx, suggesting that smoking reduction leads to increased oxidation of carbohydrates. This is consistent with the findings of Hellerstein *et al.* [27] who found that in the non-

smoking phase, RQ and total carbohydrate oxidation tended to be higher as compared to the smoking state. Our finding that IMGU and GOx, but not NGU, correlates with CO and/or metabolite levels might suggest that smoking exerts a greater influence on carbohydrate oxidation than glycogen storage.

Our study has certain limitations. Our high subject non-completion rate likely led to our study being underpowered, causing many outcome measures to be non-significant. This study demanded a level of time and scheduling commitment from subjects that challenged our recruitment and retention efforts, especially given our low-income, minority population's socioeconomic constraints. Subjects also self-reduced their smoking (despite being specifically instructed not to do so) in the time interval between qualifying and completing all baseline measures, which might have reduced the magnitudes of change that could have been detected, particularly through Phase I. Although this testifies to our subjects' motivation to quit, it also shows that they were not strongly addicted to nicotine and were able to self-initiate behavioral changes. This might have been avoided had we targeted heavier smokers, but then reduction rates might have been lower with more heavily addicted smokers, which in turn might also have attenuated the magnitude of changes detected. We also did not control for any confounding effects of bupropion, particularly with respect to the Phase I changes in central fat. However, while bupropion is known to enhance

**Table 3**  
Statistically Significant Pearson Correlation Coefficients Between Smoking Severity and Metabolic Measures.

	Phase I		Phase II		Overall	
	CO	CO	Urine Metabolites	CO	Urine Metabolites	
Anthropometry						
Weight			0.50 (0.54)		0.49 (0.50)	
BMI					0.49 (0.52)	
CT Measures: Abdominal (Abd) Subcutaneous (Sc), Visceral (Visc), and Thigh Fat Areas						
Total Abd Fat		(0.52)				
Abd Visc Fat		(0.56)				
Thigh Fat	0.71 (0.72)	(0.84)				
Total Abd Fat %-to-Thigh Fat % Ratio	-0.71 (-0.71)					
Abd Sc Fat %-to-Thigh Fat % Ratio	-0.60 (-0.61)					
Abd Visc Fat %-to-Thigh Fat % Ratio	-0.67 (-0.64)					
Hepatic Glucose Output (HGO)						
HGO/Weight			0.50 (0.53)			
Peripheral Insulin-Mediated Glucose Uptake (IMGU)						
IMGU/LM (Insulin-Adjusted)		-0.51 (-0.56)				
Respiratory Quotient (RQ)						
Basal RQ			-0.49 (-0.60)			
Max RQ			-0.52 (-0.57)	-0.50 (-0.49)		
Glucose Oxidation Rate (GOx)						
Basal GOx/Weight			-0.47 (-0.54)			
Max GOx/Weight			-0.54 (-0.57)	-0.49 (-0.48)		

Values represent Pearson's correlation coefficients. Only correlations coefficients that are statistically significant ( $p < 0.05$ ) at a conservative estimate of  $r \geq \pm 0.47$  are shown. Coefficients in parentheses are those adjusted for corresponding changes in caloric intake and caloric expenditure. Abd, abdominal; BMI, body mass index; Cig, cigarette; CO, carbon monoxide; FM, fat mass; GOx, glucose oxidation rate; HGO, hepatic glucose output; IMGU, insulin-mediated glucose uptake; LM, lean mass; Max, maximum; RQ, respiratory quotient; Sc, subcutaneous; Visc, visceral.

overall weight loss [33], we did not detect any significant weight loss. In addition, Botella-Carretero *et al.* [34] previously found no difference in waist-hip ratio changes between the use of nicotine replacement and bupropion. These observations would argue against any such confounding in our study, but a direct comparison of these measures pre- and post-bupropion use alone still would have been helpful. In addition, while each insulin infusion stage of our euglycemic clamp procedure lasted 60 min instead of the traditional 90 min to ensure steady-state, the glucose infusion rates over the final 20 min of each stage did not deviate more than  $\pm 3.5\%$  from the mean of each stage. Lastly, as an exploratory study with many analyses conducted on relatively few subjects, all of the associations that we found can only suggest possible causative relationships, and will need to be more rigorously confirmed in properly designed follow-up studies.

## Conclusions

We conclude that, in human smokers who reduce their smoking,

there may be a transient worsening of central fat redistribution within 2 months, followed by a longer-term improvement with prolonged maintenance of the reduced smoking, over 24 weeks. Also, with prolonged maintenance of reduced smoking over 24 weeks, HGO may improve. Lower levels of CO and/or urine nicotine metabolites correlated independently with lower HGO, higher rates of IMGU, and greater reliance on carbohydrates as metabolic substrates. These observations should be further clarified with studies specifically examining each of these measures, and by targeting larger numbers of subjects.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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