Short-Term Alterations in Carbohydrate Energy Intake in Humans

Striking Effects on Hepatic Glucose Production, De Novo Lipogenesis, Lipolysis, and Whole-Body Fuel Selection

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Abstract

Short-term alterations in dietary carbohydrate (CHO) energy are known to alter whole-body fuel selection in humans, but the metabolic mechanisms remain unknown. We used stable isotope-mass spectrometric methods with indirect calorimetry in normal subjects to quantify the metabolic response to six dietary phases (5 d each), ranging from 50% surplus CHO (+50% CHO) to 50% deficient CHO (−50% CHO), and 50% surplus fat (+50% fat). Fasting hepatic glucose production (HGP) varied by > 40% from deficient to surplus CHO diets (1.78±0.08 vs 2.43±0.09 mg/kg per min, $P < 0.01$). Increased HGP on surplus CHO occurred despite significantly higher serum insulin concentrations. Lipolysis correlated inversely with CHO intake as did the proportion of whole-body lipolytic flux oxidized. Fractional de novo hepatic lipogenesis (DNL) increased more than 10-fold on surplus CHO and was unmeasurable on deficient CHO diets; thus, the preceding 5-d CHO intake could be inferred from DNL. Nevertheless, absolute hepatic DNL accounted for < 5 g fatty acids synthesized per day even on +50% CHO. Whole-body CHO oxidation increased sixfold and fat oxidation decreased > 90% on surplus CHO diets. CHO oxidation was highly correlated with HGP ($r^2 = 0.60$). HGP could account for 85% of fasting CHO oxidation on +25% CHO and 67% on +50% CHO diets. Some oxidation of intracellular CHO stores was therefore also occurring. +50% fat diet had no effects on HGP, DNL, or fuel selection.

We conclude that altered CHO intake alters HGP specifically and in a dose-dependent manner, that HGP may mediate the effects of CHO on whole-body fuel selection both by providing substrate and by altering serum insulin concentrations, that altered lipolysis and tissue oxidation efficiency contribute to changes in fat oxidation, and that surplus CHO is not substantially converted by the liver to fat as it spares fat oxidation, but that fractional DNL may nevertheless be a qualitative marker of recent CHO intake. (*J. Clin. Invest.* 1995. 96:2735–2743.) Key words: carbohydrate intake • nutrient balances • de novo lipogenesis • fuel selection • hepatic glucose production

Introduction

Short-term alterations in energy intake induce a number of metabolic and hormonal changes in humans (1–6). The most extensively documented consequence of surplus energy intake is the effect of excess carbohydrate (CHO)[1] on whole body fuel selection. Several authors (4–6) have reported that the addition of surplus CHO energy to a mixed diet results in nearly complete replacement of fat by carbohydrate oxidation in the whole body, when measured by indirect calorimetry. The metabolic mechanisms responsible for the ability of dietary CHO to influence fuel selection and its own oxidation (7–9) remain unknown, however. One question is the metabolic source of the CHO oxidized by tissues, particularly in the fasted state. Stimulation of CHO oxidation after high CHO meals is not surprising, since increases in glucose and insulin concentrations suppress oxidation of fat and increase oxidation of CHO (10, 11). How CHO replaces fat in the tissue fuel mixture in the postabsorptive state is less obvious, since fat generally represents the major fuel consumed in the postabsorptive state. One possibility is that the oxidized CHO comes from plasma glucose produced by the liver; alternatively, it may derive from stored glycogen in tissues such as muscle. Along the same lines, fat oxidation may be reduced due to inhibited lipolysis or due to reduced tissue fatty acid (FA) extraction and oxidation without a change in lipolysis (12, 13). Another area of uncertainty is whether surplus CHO intake stimulates de novo lipogenesis (DNL) in humans. Most short-term studies (4, 7, 8, and 14) of CHO addition to mixed diets, even in large quantities, have failed to observe net DNL, based on indirect calorimetry. Indirect calorimetry cannot exclude concurrent DNL and fat oxidation, however. This would be counted as simple CHO oxidation by gas exchange techniques. The DNL/fat oxidation cycle would contribute to an apparent stimulation of CHO oxidation if it occurred.

With regard to short-term energy deficiency, the effects on plasma glucose metabolism are of particular interest. In non–insulin-dependent diabetes mellitus (NIDDM), marked reductions in plasma glucose, insulin, and triglyceride (TG) concentrations occur within a few days of initiating energy-restricted

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1. *Abbreviations used in this paper:* BP, binding protein; CHO, carbohydrate; −25 and −50% CHO, dietary CHO 25 and 50% deficient, respectively; 25 and 50% CHO, 25 and 50% excess dietary CHO, respectively; DNL, de novo lipogenesis; +50% fat, 50% excess dietary fat; FA, fatty acid; HGP, hepatic glucose production; M_0, mass + 0; m/z, mass/charge; NIDDM, non–insulin-dependent diabetes mellitus; NPRQ, nonprotein RQ; Ra, rate of appearance; TG, triglyceride.

diets (15–17), and the early response to diet predicts the long-term metabolic response to weight loss (18). The metabolic mechanisms underlying changes in glycemia during short-term energy restriction are not well-established, however. Fasting-induced fall in blood glucose in NIDDM is due to reduced hepatic glucose production (HGP) rather than improved insulin secretion or peripheral glucose use (19). We recently observed in normal rats that a 24-h fast reduces HGP by 50%, due entirely to an 80% decrease in the input from liver glycogen with no change in absolute gluconeogenesis (20). Reduction in hepatic glycogen content may therefore directly influence HGP or its sensitivity to suppression by insulin, but whether HGP is sensitive to CHO balance in normal humans remains uncertain. This is particularly intriguing in light of recent reports demonstrating relative constancy of HGP in the face of acute gluconeogenic substrate loads in humans (21, 22). One possibility is that the hormonal and autoregulatory mechanisms (21) that maintain HGP constant despite large substrate loads do not defend HGP against changes in CHO balance. This model has not been specifically tested in humans, however.

Our objective in this study was to determine the intermediary metabolic effects of short-term alterations in CHO or fat energy intake in normal humans, and their relationship to whole-body fuel selection. The following specific questions were addressed: (*a*) what is the source of the CHO oxidized by tissues in the fasted state in response to short-term increases in CHO intake (HGP and/or tissue carbohydrate stores) and do changes in the production rate of circulating substrates (HGP and lipolysis) correlate with changes in net whole-body fuel use? (*b*) Are the intermediary metabolic effects of altered CHO intake dose related and specific for CHO? (*c*) Does short-term surplus CHO intake stimulate quantitatively substantial hepatic DNL or is fat storage not an important fate of surplus CHO calories? (*d*) Does sensitivity of fasting HGP to suppression by insulin change in response to short-term alterations in energy intake? (*e*) Are any metabolic measurements sufficiently sensitive and specific to be useful as biomarkers of recent CHO energy intake?

Methods

Human subjects. Volunteers were recruited by advertisement. Protocols received prior approval from the University of California at San Francisco Committee on Human Research and subjects gave written informed consent before enrollment. Six normal volunteers were enrolled. Subjects had no history of medical illnesses, showed no abnormalities on screening physical examination or by laboratory testing, were < 120% ideal body weight, had stable weight over the preceding 6 mo, had normal serum glucose and lipids, and had a fasting serum insulin concentration < 15 μU/ml. Subjects were also documented to be human immunodeficiency virus seronegative, because metabolic abnormalities have been documented in asymptomatic seropositivity (23). Use of medications with potential metabolic effects (e.g., β-blockers, theophylline, diuretics, glucocorticoids, β-agonists, phenytoin) was excluded. The mean age of the subjects was 35±3 yr, weight was 74.5±5.2 kg, height was 175±3 cm, and body mass index was 24.2±1.2 kg/m^2 (mean±SE).

Study design. Subjects participated in two separate 15-d inpatient metabolic ward studies in the General Clinical Research Center of San Francisco General Hospital. During the first admission, subjects were placed on sequential 5-d dietary phases. The first 5 d consisted of a eucaloric diet of the patient's usual macronutrient composition, designed to be weight maintaining. Initial estimated energy requirements were based on Harris-Benedict equations, with adjustments by the dietician if 3-d dietary recalls suggested higher or lower routine daily intakes. Food intake was further adjusted over the first 72 h on the metabolic ward based on the subject's report of hunger and/or changes in weight, up to a maximum of 500 kcal/d above or below predicted energy needs. Diets were prepared in the metabolic kitchen. Body weights were stable over the 5-d eucaloric phase (< 1 kg change in all subjects). The second 5-d period consisted of 50% extra energy added to the diet, predominantly as CHO (+50% CHO, Table I). This represented a change from 324±18 to 684±39 g CHO/d, or an extra 360 g CHO added/d. In some subjects, 5–10% extra fat energy (~ 30 g fat) and 5% extra protein energy (~ 35 g protein) had to be added as well, to maintain palatability. The third 5-d diet period contained 50% reduction in energy, predominantly as CHO (−50% CHO, Table I). This represented a reduction to 114±12 g CHO/d, or 210 g fewer CHO/d than in the eucaloric phase. Again, some reduction in fat and protein intake occurred (43 g fat and 30 g protein reduced vs eucaloric phase). On the fifth day of each dietary phase, a metabolic infusion study was performed (see below). The subject continued to ingest the diet of that particular phase during the metabolic infusion study day. Subjects returned for a second admission after at least 2 wk at home. The dietary phases during this admission were, sequentially, 25% energy excess as CHO (+25% CHO) with no significant changes in fat or protein intake compared to eucaloric, 25% energy deficiency as CHO (−25% CHO) again with no significant changes in fat or protein intake, and 50% energy excess as fat (+50% fat). The repeat study was otherwise identical to the first admission. Actual intake of energy and protein in each of the dietary phases is shown (Table I).

Metabolic infusion studies were designed to quantify several metabolic processes (13): HGP, hepatic DNL, adipose lipolysis, whole-body fat oxidation, whole-body CHO oxidation, energy expenditure, nonoxidative glucose disposal and whole-body reesterification of FFA (Fig. 1). The studies consisted of intravenous infusions of 6,6-d_2-glucose (0.05 mg/kg per min) from 6:00 a.m.–10:00 a.m., d_5-glycerol (9.7 μg/kg per min) from 6:00 a.m.–10:00 a.m., and sodium [1-^{13}C]-acetate (0.120 mmol/kg per h) from 2:00 a.m.–6:00 p.m. Indirect calorimetry was performed in fasted and fed states (between 7:00 a.m. and 9:00 a.m. and between 3:00 p.m. and 5:00 p.m.). Three 30-min measurements were performed during the 2-h time block in the morning and two 30-min periods in the afternoon, using a Deltatrac Metabolic Cart (Sensor Medix, Yorba Linda, CA) in the hooded mode. Measured daily protein intake was used to estimate protein oxidation for calculation of nonprotein respiratory quotient (NPRQ) (12), since nitrogen balance is achieved in humans within ~ 72 h of altering dietary protein or energy intake (24).

Subjects remained fasted (other than noncaloric, non–caffeine-containing fluids) from 10:00 p.m. of the preceding evening until 10:00 a.m. of the study day, then consumed the usual diet of their current study phase from 10:00 a.m. through the end of the day. A baseline blood draw was taken the prior evening and repeat samples were taken from an indwelling blood-drawing line at 8:00 a.m., 9:00 a.m., 9:30 a.m., 9:50 a.m., 10:00 a.m., 12:00 noon, 2:00 p.m., 4:00 p.m., and 6:00 p.m.

Clinical laboratory measurements. Serum lipids were measured by standard methods (MetWest Laboratories, San Francisco, CA). Serum insulin was measured by RIA, and serum glucose by autoanalyzer. IGF-1 and binding protein-3 (BP-3) were measured by immunoassay as described previously (25).

Mass spectrometry. Gas chromatography mass-spectrometry (model 5971; Hewlett-Packard Co., Palo Alto, CA) was used for analysis of isotopic enrichments of serum glucose, glycerol, and fatty acid-methyl esters from VLDL. Glucose and glycerol were isolated from plasma as described previously (13). Glucose was converted to the pentaacetate derivative and analyzed using a Duroband (DB)-17, 60-m, 0.25-mm interior diameter fused silica column, under selected ion monitoring with chemical ionization. Abundances of ions at mass/charge (m/z) 331–333 were quantified, and enrichments were determined by comparison to standard curves of 6,6-d_2-glucose. Glycerol-triacetate was analyzed with a DB-225 column, using chemical ionization (12). Selected ion monitoring was used at m/z 159 and 164, representing mass + 0

Table I. Dietary Energy and Protein Intakes on Different Diet Phases

	Diet phase					
	Eucaloric	+50% CHO	−50% CHO	+25% CHO	−25% CHO	+50% Fat
Energy intake (kcal/d)	2,723±129*	4,550±277‡	1,406±80§	3,484±236‖	2,061±135¶	4,236±267‡
CHO intake (g/d)	324±18*	684±39‡	114±12§	494±35‖	149±13§	319±27*
Percent kcal	47±1*	59±1	32±2	56±1	29±1	30±1
Fat intake (g/d)	117±6*	148±12*	74±4‡	123±9*	122±7*	272±16§
Percent kcal	38±1	29±1	47±2	31±1	53±1	57±1
Protein intake (g/d)	104±6*	141±11‡	74±6§	114±8*‡	96±8*§	139±12*‡
Percent kcal	15±0.4	12±0.5	21±1	13±0.3	18±1	13±1

Experimental details are described in the text. Data are expressed as mean±SEM. Values in the same row not sharing a common superscript are significantly different ($P < 0.05$). Percent kcal, percent total energy intake.

(M_0) and M_5, and enrichments were determined by comparison to standard curves of d_5-glycerol. For fatty acid methyl esters analyses, a 20-m fused DB-1 silica column was used (isothermal at 200°C), with electron impact ionization (26, 27). Ions at m/z 270–272 were monitored, representing the parent M_0 through the M_2 isotopomers.

Calculations. The rate of appearance (Ra) glycerol and Ra glucose were calculated by the dilution technique (13). HGP was considered to be identical to Ra glucose under fasting conditions.

Fractional DNL was calculated by the mass isotopomer distribution analysis technique, as described in detail elsewhere (26–28). The ratio of excess double-labeled to excess single-labeled species (EM_2/EM_1) in VLDL-palmitate reveals the isotopic enrichment of the true precursor for lipogenesis (hepatic cytosolic acetyl-CoA) by application of probability logic based on the binomial expansion. The fractional contribution from DNL to the VLDL fatty acid can then be calculated by the precursor–product relationship (26, 28).

On most −50% CHO diets and some −25% CHO diets, incorporation into VLDL-palmitate was too low for analytically reliable calculations of true precursor enrichment. A value of < 0.5 M percent excess in palmitate M_2 (m/z 272) was used as the cutoff. In these cases, an acetyl-CoA precursor enrichment of 3 M percent excess (0.03 M excess) was used for calculations of fractional DNL, since this represents a rough lower limit of precursor enrichments achieved in these and other human subjects infused with [^{13}C]acetate at this rate (23, 26–29). Estimated values for fractional DNL under conditions of low incorporation would therefore err on the side of over-estimating fractional DNL, if precursor enrichments were in fact higher than the minimal values used.

Absolute DNL was estimated by two methods, both based on multiplication of VLDL-TG production rate times fractional DNL. By one method, we used an estimated VLDL-TG production rate of 30 g/d (13). This value was multiplied times mean daily fractional DNL to calculate absolute DNL, as described previously (13, 26). The second method was used only in the +50% CHO phase, during which period isotopic incorporation into VLDL-TG was high enough for kinetic modeling of the rate constant of rise toward plateau and direct calculation of VLDL-TG turnover (28). Fractional DNL results from hourly blood samples drawn between 4:00 and 10:00 a.m. were plotted, and the best fit for the curve of DNL vs time was calculated using the DeltaGraph Pro 3.5 computer program (DeltaPoint, Monterey, CA). The data was fit to the equation $y = A_0 \cdot (1\text{-}e^{-K_s(t-c)})$ where y = DNL, A_0 = the plateau value of DNL, $K_s(h^{-1})$ = the rate constant of rise to plateau in VLDL-TG, t is in hours, and c is the lag period before isotope incorporation into secreted VLDL-TG. The half-life of VLDL-TG in plasma was calculated as, $t_{1/2}$ (h) = $0.693/K_s$. Absolute DNL was then calculated as, Absolute DNL (g/d) = K_s (h^{-1}) × 24 h/d × pool size (g), where pool size = TG concentration (mg/dl) × estimated plasma vol (dl). Plasma vol was estimated to be 3 liters (~ 4% of body wt).

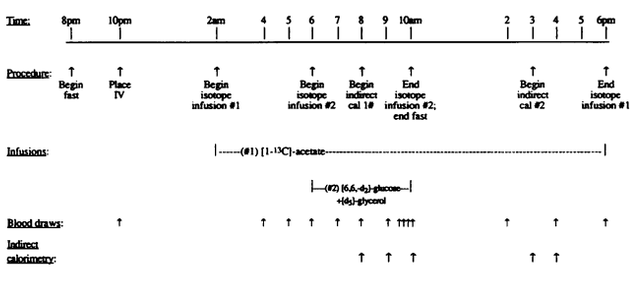

Figure 1. Metabolic infusion protocol, performed on days 5, 10, and 15 of each General Clinical Research Center admission. *indirect cal,* indirect calorimetry.

For simplicity in calculating 24 h absolute DNL, the plateau fasting values of DNL were assumed to represent 12 h of the d, and the maximal fed value of DNL the remaining 12 h of the d (i.e., their mean value was used). We also assumed that the fractional DNL values for VLDL-palmitate represent all nonessential fatty acids (stearate, oleate, etc.) present in VLDL, realizing that this will overestimate absolute DNL to the extent that de novo synthesis of stearate or oleate are less than de novo synthesis of palmitate, which we have elsewhere observed to be the case (26, 27). Thus, our calculations were intended to err on the side of overestimation of DNL (i.e., to avoid underestimating absolute hepatic DNL).

NPRQ, energy expenditure, and whole-body oxidation of fat and carbohydrate were calculated by indirect calorimetry according to standard equations (30). Whole-body reesterification of fatty acids was calculated as the difference between adipocyte lipolysis (Ra glycerol × 3 × average mol wt of fatty acid, assumed to be 270 D) and whole-body fat oxidation, with both expressed as milligrams per kilogram per minute. This calculation reflects both intraadipocyte and extraadipocyte fatty acid reesterification (12, 13).

Statistical analyses. Effects of dietary phase were determined by repeated measures ANOVA, with a procedurewise error rate of 5% (i.e., significance at 0.05). Correlation coefficients were determined by least squares linear regression analysis.

Results

Effects of alterations in energy intake on body weight, NPRQ, whole-body fat oxidation, CHO oxidation, and energy expenditure. Body weight changed in parallel with energy intake (Table II). On the +50% CHO and +50% fat, weight gain was 2.3±0.2 and 2.1±0.4 kg, both significantly different from eucaloric value ($P < 0.01$) but not different from each other. On the −50% CHO diet, weight loss was 2.8±0.4 kg.

Table II. Whole-Body Fuel Use, Body Weight, and Intermediary Metabolism on Different Diets

Parameter	Dietary phase (mean ± SEM)					
	Eucaloric	+50% CHO	−50% CHO	+25% CHO	−25% CHO	+50% Fat
Energy expenditure						
REE (kcal/kg per min)	0.0166±0.0007*	0.0174±0.0005*	0.0171±0.0007*	0.0163±0.0005*	0.0168±0.0007*	0.0166±0.0011*
NPRQ	0.84±0.004*	0.95±0.01‡	0.77±0.01§	0.91±0.01‖	0.80±0.004*§¶	0.84±0.02*
Body weight change (kg)	+0.5±0.2*	+2.3±0.2‡	−2.8±0.4§	+1.1±0.1‖	−1.9±0.3§	+2.1±0.4‡
Fat metabolism						
Lipolysis (mg/kg per min)	1.31±0.17*‡	0.81±0.08§	1.69±0.13*	0.76±0.10§	1.10±0.15‡	0.74±0.06§
Fat oxidation (mg/kg per min)	0.79±0.05*	0.11±0.08‡	1.25±0.03§	0.35±0.14‡¶	1.04±0.07‖	0.84±0.28*§‖¶
FA reesterification (mg/kg per min)	0.52±0.19*‡	0.70±0.13*	0.44±0.12*‡	0.39±0.05*	0.06±0.16*	−0.10±0.29*‡
Lipolytic flux oxidized (%)	60±5*‡	14±2§	74±5*‖	46±6‡	95±6‖	113±27‡‖
CHO metabolism						
Ra glucose (mg/kg per min)	2.15±0.06*	2.43±0.08‡	1.78±0.08§	2.29±0.06*‡	1.91±0.07§	2.25±0.08*‡
CHO oxidation (mg/kg per min)	1.68±0.13*	3.62±0.21‡	0.64±0.16§	2.70±0.18‖	1.10±0.12§	1.61±0.41*
Nonoxidative glucose disposal (mg/kg per min)	0.47±0.13*	−1.19±0.16‡	1.14±0.19§	−0.41±0.17‖	0.83±0.15*§	0.63±0.40*§

Methods and calculations are described in the text. Fat metabolism parameters are expressed as milligrams FA/kg per min, assuming an average FA of 270 D. Values in the same row not sharing a superscript are significantly different ($P < 0.05$). Lipolytic flux oxidized is calculated as the ratio of whole-body fat oxidation to whole-body lipolysis, expressed as a percentage. REE, resting energy expenditure.

In the overnight-fasted state, changes in CHO energy intake altered NPRQ (Table II). NPRQ varied between 0.77±0.02 and 0.95±0.03 ($P < 0.01$) on diets ranging from −50% CHO to +50% CHO, respectively. Whole-body oxidation of CHO and fat (Table II) were also closely associated with dietary CHO, varying over a sixfold range (0.64±0.16–3.62±0.21 mg/kg per min, for CHO oxidation, $P < 0.001$, and 0.11 to 1.25 mg/kg per min for fat oxidation, $P < 0.001$), but there were no effects of +50% fat diet on fuel selection when compared to eucaloric diet. No significant changes in energy expenditure were observed during fasting on any diet (Table II). Thus, fuel selection rather than energy expenditure were affected by altered dietary CHO in the fasted state.

In the fed state (~ 3 h after lunch), nonprotein RQs were 0.87±0.03 (eucaloric), 0.96±0.04 (+50% CHO), 0.76±0.02 (−50% CHO), 0.93±0.02 (+25% CHO), 0.78±0.03 (−25% CHO), and 0.84±0.02 (+50% fat). These RQ values were remarkably similar to RQ values during the overnight-fasted state on each diet (Table II). Energy expenditure was higher in the fed state compared to the fasted state under all dietary conditions, ranging from an 11% increase (−25% CHO) to 29–32% increase (+50% CHO and +50% fat). Fed-state energy expenditure was 11% higher during +50% CHO compared to −50% CHO diet (1.68±0.12 vs 1.49±0.05 kcal/min, $P < 0.05$).

Effects on HGP, nonoxidative glucose disposal, and serum insulin concentrations. Fasting HGP responded in a linear and statistically significant manner to changes in CHO intake but did not change in response to increased dietary fat (Fig. 2, *A* and *B*). The difference in HGP between −50% CHO and +50% CHO was 41% (1.76±0.09 vs 2.48±0.13 mg/kg per min, $P < 0.01$). Nonoxidative glucose disposal (HGP minus CHO oxidation) also showed marked changes (Table II). Nonoxidative glucose disposal became a negative number on surplus CHO diets (−1.19±0.16 mg/kg per min on +50% CHO, −0.41±0.17 mg/kg per min on +25% CHO), indicating that intracellular CHO was being oxidized in addition to oxidation of glucose derived from plasma. Nonoxidative glucose disposal was significantly higher on carbohydrate-deficient diets (Table II) in association with increased oxidation of fat, presumably representing glycolytic recycling (the glucose-fatty acid cycle of Randle, reference 31). No significant differences in fasting glucose concentrations were noted (not shown). Although fasting serum insulin concentrations remained within the normal

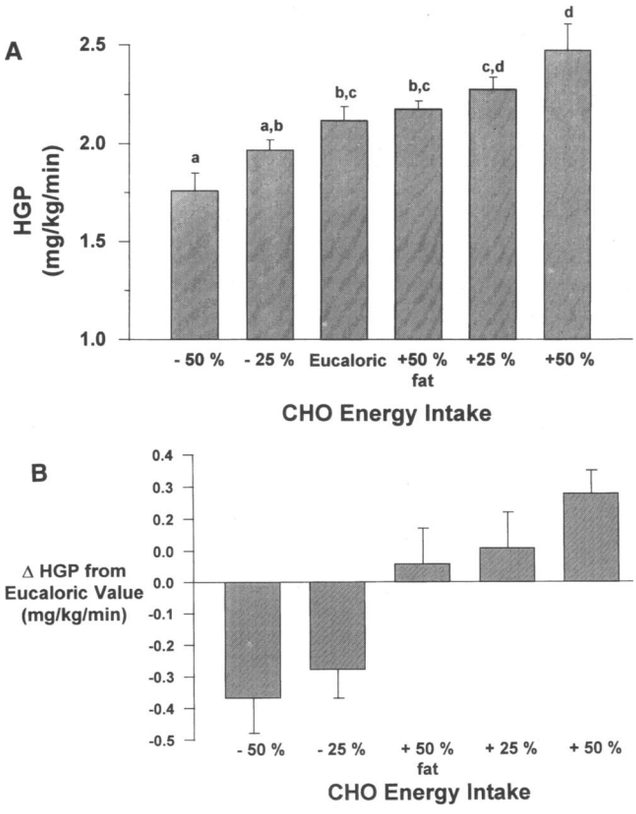

Figure 2. Fasting HGP or Ra glucose in response to short-term alterations in dietary energy content. Methods and calculations are described in the text. Values not sharing a common superscript are significantly different ($P < 0.05$). (*A*) HGP (mg/kg per min). (*B*) HGP (change from eucaloric value, mg/kg per min).

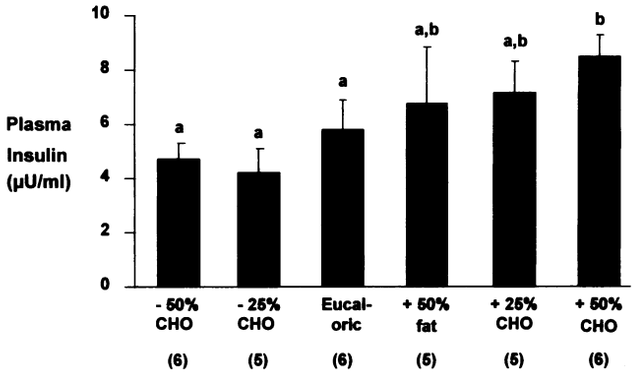

Figure 3. Serum insulin concentrations in response to short-term alterations in dietary energy content. Values not sharing a common superscript are significantly different ($P < 0.05$).

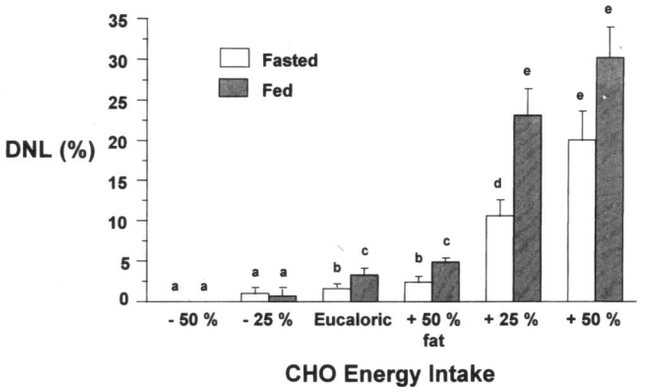

Figure 5. Hepatic DNL in fasted and fed states in response to short-term alterations in dietary energy content. Fractional contribution from DNL to palmitate isolated from circulating VLDL fatty acids was determined as described in the text. Values not sharing a common superscript are significantly different ($P < 0.05$).

range (< 15 μU/ml) on all diets, the levels were significantly altered by CHO intake (Fig. 3). The eucaloric values for fasting insulin concentrations were 5.8±1.1 μU/ml. These rose to 7.2±1.1 μU/ml and 8.5±0.8 μU/ml on +25% CHO and +50% CHO respectively, fell to 4.2±0.9 and 4.7±0.6 μU/ml on −25% CHO and −50% CHO diets, respectively, and were 6.8±2.0 μU/ml on +50% fat diets. Both surplus CHO diets were significantly different for insulin concentration than both deficient CHO diets ($P < 0.05$), and +50% CHO was significantly different from eucaloric diet ($P < 0.05$). The combination of increased serum insulin concentrations and increased HGP on surplus CHO diets represents, by definition, a form of insulin resistance, in this case induced by only 5 d of surplus CHO energy intake. Conversely, improved sensitivity of HGP to suppression by insulin was documented in response to 5 d of deficient CHO energy intake, since HGP was reduced despite reductions in serum insulin concentrations. The dose-response curve for HGP as a function of serum insulin concentrations was not studied, however. We cannot therefore say whether the curve for insulin suppression of HGP was simply shifted to the right or had a different shape.

Effects on lipolysis and reesterification. Plasma Ra glycerol reflects absolute adipose lipolysis (12, 13), since glycerol released from intracellular hydrolysis of TG cannot be reesterified in the adipocyte. Fasting Ra glycerol was inversely related to dietary CHO intake although there was greater variability in the results than for other parameters (Fig. 4). Ra glycerol fell by > 50% on +50% CHO compared to −50% CHO (0.09±0.01 vs 0.19±0.01 mg/kg per min, $P < 0.001$). Whole-body oxidation of fat varied even more robustly than Ra glycerol (Table II), decreasing by 90% (0.03±0.02 vs 0.34±0.01 mg/kg per min, $P < 0.01$). The +50% fat diet reduced lipolysis but not whole-body fat oxidation. Whole-body reesterification of fatty acids, representing both adipocyte and hepatic reesterification (12, 13), was calculated by the difference between absolute lipolysis and whole-body fat oxidation (both expressed as milligrams per fat kilogram per minute, Table II). The whole-body reesterification rate was not significantly different on any of the diets, although there was a trend to lower values on −25% CHO and +50% fat diets. Because whole-body fat oxidation varied greatly while whole-body reesterification did not, the proportion of lipolytic flux oxidized changed substantially in response to diet (Table II). Eucaloric values of 54±6% fell to as low as 12±2% on surplus CHO diets and rose to as high as 85±7% on deficient CHO diets. Thus, tissue oxidation efficiency of FA may also play a role, along with lipolysis, in the altered fat oxidation induced by changes in dietary CHO.

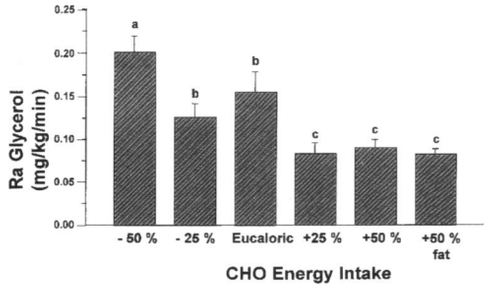

Figure 4. Ra glycerol (lipolysis) in response to short-term alterations in dietary energy content. Values not sharing a common superscript are significantly different ($P < 0.05$).

Hepatic DNL. The most robust, sensitive, and specific change observed in response to altered CHO intake was in fractional DNL (Fig. 5). Excess CHO intake increased fractional DNL by 6- to 10-fold, from eucaloric diet values of 1.6±0.6/3.3±0.8% (fasted/fed values) to 10.6±2.0/23.2±3.3% and 20.1±3.6/30.4±3.7% (+25% CHO and +50% CHO, respectively, $P < 0.01$ for each vs eucaloric values). Deficient CHO diets reduced fractional DNL to unmeasurably low values (estimated to be 0.1/0.1% and 1.0±0.6/0.7±0.7%, on −50% CHO and −25% CHO diets, respectively). The values of fractional DNL during deficient CHO diets were significantly lower than fractional DNL on eucaloric diets ($P < 0.05$).

Absolute rates of DNL were not quantitatively large even on excess CHO diets, however. Whether calculated from estimated VLDL-TG production rates (13) or from kinetic modeling of VLDL-TG isotope incorporation (28), absolute 24-h hepatic DNL remained < 5 g fat synthesized per day (Table III), even though our calculations are likely to somewhat overestimate absolute DNL (see above). Since NPRQ was < 1.0 in these subjects in both the fasted and fed states; substantial DNL was neither occurring in the whole body (in a net sense) nor in the liver (in an absolute sense).

Table III. Estimates of Absolute DNL during +50% CHO Diet

Subject	Method 1							Method 2	
	Mean fractional DNL	K_s	$t_{1/2}$	TG concentration	Calculated TG production rate	Absolute DNL	Glucose converted to fat	Absolute DNL	Glucose converted to fat
	%	h^{-1}	h	mg/dl	g/d	g/d	g/d	g/d	g/d
1	22.8	0.363	1.91	133	34.8	3.6	10.0	3.1	8.7
2	37.4	0.463	1.50	94	31.3	5.3	14.8	5.0	14.2
3	15.9	0.413	1.68	91	27.1	1.9	5.4	2.1	6.0
4	21.2	0.242	2.86	112	19.5	1.9	5.2	2.9	8.1
5	25.2	0.170	4.01	83	10.2	1.2	3.3	3.4	9.6
6	30.1	0.568	1.22	110	45.0	6.1	17.3	4.1	11.5
Mean±SE	25.4±3.1	0.370±.059	2.21±.44	104±7	28.0±5.0	3.3±.8	9.3±2.3	3.4±.4	9.7±1.2

Method 1, absolute DNL calculated from measured VLDL-TG production rate, based on kinetic modeling from [^{13}C]acetate isotope incorporation curve, as described in the text. TG production rate during overnight fasting is extrapolated to 24 h. Method 2, absolute DNL calculated from an estimated maximal VLDL-TG production rate of 30 g/d (26, 29). For both Methods 1 and 2, mean fractional DNL was calculated by assuming the plateau fasted value to represent 12 h of the day and the maximal fed values of DNL to represent the remaining 12 h of the day (i.e., the average of fasted and fed plateau values was used). Glucose conversion to fat was calculated as 2.8 g glucose/g fatty acid, based on the approximate stoichiometry of DNL (14).

Correlations among intermediary metabolic and fuel use parameters. Correlations among metabolic and fuel selection parameters were determined (Table IV). HGP had strong positive correlations with whole-body CHO oxidation, DNL, and an inverse correlation with fat oxidation. Ra glycerol was less strongly correlated with whole-body fat oxidation. DNL was strongly correlated with whole-body fuel oxidation rates (fat oxidation and CHO oxidation). Serum insulin concentrations correlated directly but weakly with CHO oxidation, and HGP and insulin correlated inversely but weakly with Ra glycerol. Whole-body fat oxidation and CHO oxidation were strongly inversely correlated, as expected.

Other laboratory results. No significant differences in fasting serum glucose or triglyceride concentrations were observed between dietary phases (not shown). Serum IGF-1 and IGF BP-3 concentrations also did not vary significantly among any dietary phases (Table V).

Discussion

5 d of altered energy intake results in only modest changes in the stored energy content of the body. A 25–50% excess or deficit of energy per day represents a maximum change of ~ 4,000–8,000 kcal in the body over this period of time, but when in the form of CHO energy, the metabolic consequences that we observed were striking, particularly for HGP and fractional hepatic DNL. Moreover, for each of the parameters that we measured there was a graded, dose-response relationship with the CHO energy content of the diet (Figs. 2–5), and there was little apparent effect of the fat energy content of the diet on intermediary metabolism or whole-body fuel selection. These intermediary metabolic measurements provide insight into the metabolic mechanisms underlying the dietary CHO effects on whole-body fuel selection (7–9).

Our first question was the source of CHO oxidized in the fasted state on surplus CHO diets, since whole-body CHO oxidation is markedly stimulated under these conditions. The results show clearly that HGP increases in proportion to dietary CHO (Fig. 2, Table II), and that HGP correlates strongly with whole-body CHO oxidation (Table IV), but that oxidation of intracellular CHO stores also increased, as reflected in negative values of nonoxidative glucose disposal (whole-body CHO oxidation values that were greater than HGP, Table II). HGP could provide a maximum of 2.43 mg/kg per min out of a total CHO oxidation rate of 3.62 mg/kg per min (67%) on +50% CHO diet and 2.29 mg/kg per min out of 2.70 mg/kg per min (85%) on +25% CHO diet. The negative values for nonoxidative glucose disposal represent minimum estimates of intracellular CHO

Table IV. Correlation Coefficients among Parameters

	CHO oxidation	Fat oxidation	HGP	Ra glycerol	DNL	Insulin concentration
CHO oxidation	—	−0.942	0.773	−0.347	0.819	0.361
Fat oxidation	−0.942	—	−0.730	0.403	−0.781	−0.201
HGP	0.773	−0.730	—	−0.407	0.598	0.396
Ra glycerol	−0.347	0.403	−0.407	—	−0.286	−0.397
DNL	0.819	−0.781	0.598	−0.286	—	0.421
Insulin concentration	0.361	−0.201	0.396	−0.397	0.421	—

Correlations (r) were determined by univariate linear regression analysis.

Table V. Serum IGF-1 and BP-3 Levels

Dietary phase	IGF-1	IGF BP-3
	ng/ml	*ng/ml*
Eucaloric	94±13	1,846±487
+50% CHO	67±14	1,633±353
−50% CHO	87±15	1,585±209
+25% CHO	84±17	1,714±545
−25% CHO	95±25	1,642±569
+50% Fat	76±20	2,082±474

Values shows are means±SEM. Measurements were made as described elsewhere (25). There are no significant differences between any of the groups.

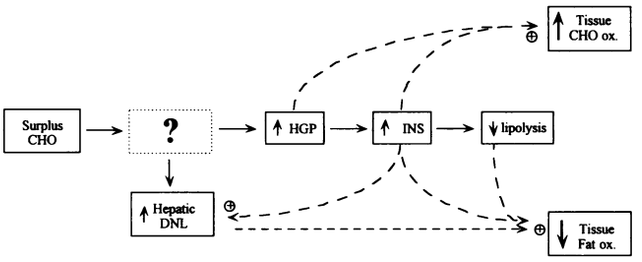

Figure 6. Hypothetical model of metabolic mechanisms responsible for sensitivity of whole-body fuel selection to dietary CHO intake. All processes other than box labeled *?* were measured. *INS,* insulin; *ox,* oxidation.

contribution to whole-body CHO oxidation. The contribution from intracellular CHO stores is a minimum estimate because, if a portion of plasma glucose disappearance is still nonoxidative, the oxidation of intracellular CHO stores would be proportionately increased. We also observed markedly reduced fasting lipolysis (Fig. 4) correlating negatively with serum insulin concentrations and HGP (Table IV). A primary increase in HGP could therefore reduce the availability of fatty acids to tissues by stimulation of pancreatic insulin secretion.

The second question that we asked was whether the metabolic response to altered CHO was graded and specific for CHO. Our results clearly show that for HGP, lipolysis, DNL, whole-body fat oxidation, whole-body CHO oxidation, and serum insulin concentrations (Figs. 2–5, Table II), the metabolic effects of altered CHO are both dose related and specific for CHO. Surplus dietary fat at the same energy content as the highest CHO diet had little or no effect on intermediary metabolism or fuel selection. It is also worth noting that the metabolic signals of altered CHO intake are short-lived. We found no evidence for carry-over effects after 5 d (i.e., metabolic "memory"). That is, the sequence eucaloric/+50% CHO/−50% CHO did not result in eucaloric values of DNL at the end of the final phase, despite the fact that net energy and CHO balances over the preceding 15 d were 0 (neutral). Instead, an underfed pattern was apparent for DNL, HGP, lipolysis, serum insulin, whole-body fuel selection, etc., on the final day of the third dietary phase. The most recent 5-d diet period is apparently what was sensed. Finally, it should be pointed out that the overfeeding pattern observed during surplus CHO diets can not be attributed to reduced activity or sedentary life-style associated with living on a metabolic ward, since there was no increase in DNL during the eucaloric phase or the +50% fat phase.

The third question that we asked was whether hepatic DNL is quantitatively important when NPRQ is less than 1.0. Our results do not support a quantitatively important rate of hepatic DNL, even on massively increased CHO intake, in these short-term studies with mixed (fat-containing) diets. Total fat synthesis was always estimated to be < 5 g/d, by two calculation methods. Thus, surplus CHO calories taken during the day are not primarily stored as fat but must be stored as glycogen, in normal humans. Rather than resulting in a diurnal cycle of fuel selection (with daytime fat synthesis in the fed state followed by nighttime fat oxidation in the fasted state), surplus CHO calories instead cause an extension of the daytime-fed metabolic pattern into the fasted period (high fasting CHO oxidation, HGP and insulin concentrations, and suppressed lipolysis). The consequence is a less cyclic diurnal fuel selection pattern rather than a cyclic one. These findings parallel our previous observations in subjects who were allowed to overeat on ad lib. diets (29), in whom absolute hepatic DNL was also < 10 g/d despite up to 3,500–4,000 kcal CHO/d and 5,500–6,000 kcal total energy/d. It should be pointed out that the occurrence of adipocyte DNL was not excluded by our studies, although the liver is generally believed to be the site of most fat synthesis in humans (32). Also, even though DNL may not be a quantitative sink for excess CHO calories, DNL may serve regulatory or signal functions in the liver or other tissues, such as the control of fatty acid reesterification vs oxidation (33).

Taken together, these results provide the basis for a hypothetical model (Fig. 6) to explain the effects of CHO energy intake on whole-body fuel selection (7–9). Increased HGP not only provides extra glucose for tissues to oxidize but it stimulates a compensatory moderate hyperinsulinemia that reduces lipolysis. The combination of higher insulin concentrations, reduced fatty acid availability to tissues with increased delivery of extracellular glucose, and a lipogenic, presumably antiketogenic milieu (33) in the liver (all measured in this study), and increased intracellular glycogen stores (presumed) might explain the markedly increased tissue CHO oxidation and decreased tissue fat oxidation that we observed. The opposite sequence of events is postulated to occur in response to CHO deprivation. Thus, the liver could affect whole-body fuel selection by altering the sensitivity of HGP to suppression by insulin and by reducing β-oxidation of FFA, with only a minor effect by synthesizing new fat from CHO. This model provides a metabolic function for the surprisingly large fluctuations in HGP in response to recent CHO balance. Rather than HGP being tightly regulated, these fluctuations in HGP may be an important adaptation that allows fuel selection to respond to dietary CHO surplus or deficit. The difference between these results with 5 d of CHO intake and previous findings during acute loads of gluconeogenic precursors (21, 22) may have important physiologic implications. HGP is tightly controlled in the face of large variations in substrate loads under eucaloric conditions but apparently adapts to changes in whole-body CHO energy balances. It will be important to understand the components of this model (Fig. 6) in greater detail, particularly the quantitative importance of the individual components.

Our fourth question concerned the suppressability of HGP by insulin as a consequence of short-term CHO surplus. The presence of increased HGP despite increased serum insulin con-

centrations on surplus CHO diets represents a form of insulin resistance occurring over 5 d in previously normal subjects. Changes in HGP induced by 5 d of surplus or deficient CHO energy varied over a range of 40% (Fig. 2), which is similar to the difference between normal and NIDDM subjects (15, 34). We did not determine the mechanism responsible for altered HGP or the dose-response relationship between HGP and serum insulin concentration. Thus, we cannot state whether the insulin suppression curve is simply shifted to the right or has a different shape. In terms of biochemical mechanisms, one possibility is the known effect of glycogen content itself on the activities of glycogen phosphorylase and glycogen synthase (35). It is worth considering the consequences for whole-body nutrient balances of this type of insulin resistance. The metabolic milieu that we documented has the net effect of increasing tissue oxidation of CHO even in the postabsorptive state, and each of the intermediary metabolic alterations that we observed contributes to this end result (increased HGP, reduced lipolysis, reduced fraction of lipolytic flux oxidized, increased circulating insulin concentrations, antiketogenic state of liver). The insulin resistance of obesity, in contrast, has been hypothesized (7) to reflect at least in part a metabolic settling point that allows balance to be achieved between fat oxidation and intake, often in the context of a chronic high fat diet or low energy expenditure. Accordingly, lipolysis and fat oxidation tend to be increased rather than suppressed in the insulin resistance associated with obesity (36, 37). Whether these differences between short-term CHO overfeeding and established obesity just represent differences in time course or fundamentally distinct types of insulin resistance remains an unanswered question.

Our final question was whether any metabolic measures were useful as biomarkers of recent energy intake. DNL was markedly stimulated on surplus CHO diets and severely reduced on CHO-deficient diets (Fig. 5) and represented the most useful parameter in this regard. Fractional DNL changed in such a robust fashion in every individual that it reflected with near 100% sensitivity and specificity the most recent dietary phase. Fractional DNL therefore may be useful as an objective biomarker of recent CHO energy intake, even though it did not represent a quantitative sink for excess CHO (< 5 g/d, Table II). The changes in fractional hepatic DNL may represent a method for objectively documenting recent CHO energy balance. In contrast, serum IGF-1 and IGF BP-3 values did not change in response to these CHO energy alterations, and other measures such as HGP tended to have too great an overlap between individuals to be reliable as specific indices of recent diet.

The clinical implications of these results are not certain, but some interesting speculations can be considered. The sensitivity of HGP to recent CHO energy intake in normal subjects parallels the therapeutic effects of short-term energy restriction in NIDDM (15–19). From a research perspective, the striking effects of short-term dietary CHO energy need to be taken into account in experimental design, since the same individual after a few days of dietary CHO imbalance can manifest markedly different metabolism despite little or no change in body weight or body composition. Measurement of fractional DNL may be an objective method to evaluate the role of recent CHO energy intake in this setting.

In conclusion, 5 d of altered energy intake when in the form of dietary CHO but not fat induced remarkable changes in intermediary metabolism and substrate oxidation in normal humans. The changes were related to CHO intake as though by a graded, dose-response relationship that applied over the full range from deficient to excess CHO intake. Alterations in HGP were surprisingly robust and clearly primary responses, since they occurred despite opposing changes in insulin concentrations. The increased fasting HGP in the face of increased insulin concentrations represents a form of insulin resistance. Secondary effects of increased HGP on insulin secretion and lipolysis may contribute to the effects on whole-body fuel selection. Although absolute hepatic DNL does not appear to be an important quantitative sink for surplus CHO calories, fractional DNL is a marker of recent CHO energy intake. Understanding these normal responses to altered CHO energy intake may prove useful for rational use of dietary energy restriction in NIDDM, obesity, and hyperlipidemia.

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