Original Article

The Greater Contribution of Gluconeogenesis to Glucose Production in Obesity Is Related to Increased Whole-Body Protein Catabolism

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Obesity is associated with an increase in the fractional contribution of gluconeogenesis (GNG) to glucose production. We tested if this was related to the altered protein metabolism in obesity. GNG_{PEP} (via phosphoenol pyruvate [PEP]) was measured after a 17-h fast using the deuterated water method and ^2H nuclear magnetic resonance spectroscopy of plasma glucose. Whole-body ^{13}C-leucine and ^3H-glucose kinetics were measured in the postabsorptive state and during a hyperinsulinemic-euglycemic-isoaminoacidemic clamp in 19 (10 men and 9 women) lean and 16 (7 men and 9 women) obese nondiabetic subjects. Endogenous glucose production was not different between groups. Postabsorptive %GNG_{PEP} and GNG_{PEP} flux were higher in obese subjects, and glycogenolysis contributed less to glucose production than in lean subjects. GNG_{PEP} flux correlated with all indexes of adiposity and with postabsorptive leucine rate of appearance (R_a) (protein catabolism). GNG_{PEP} was negatively related to the clamp glucose rate of disposal (R_d) and to the protein anabolic response to hyperinsulinemia. In conclusion, the increased contribution of GNG to glucose production in obesity is linked to increased postabsorptive protein catabolism and insulin resistance of both glucose and protein metabolism. Due to increased protein turnover rates, greater supply of gluconeogenic amino acids to the liver may trigger their preferential use over glycogen for glucose production. ***Diabetes*** **55:675–681, 2006**

The increased fractional contribution of gluconeogenesis (GNG) to postaborptive endogenous glucose production (EGP) in type 2 diabetes is well established (1), but its role in obesity is less clear. Elevated GNG has also been reported in obesity but is associated with a smaller contribution of glycogenolysis (GLY), and hence with no increase in EGP, and therefore euglycemia (2–4). Thus, since hepatic autoregulation (5) is apparently intact in obese subjects, it is not immediately clear what mechanism is responsible for increased GNG. Such autoregulation does not appear to be intact in type 2 diabetes (2,3,6), but it remains uncertain whether it causes increased EGP as hyperglycemia increases (1–3,7,8). Insulin resistance is a hallmark of both type 2 diabetes and obesity, affecting not only glucose and lipid metabolism (9) but protein as well. We have shown that whole-body protein catabolism is increased in hyperglycemic type 2 diabetic people but improves when normoglycemia is achieved with insulin and oral antihyperglycemic agents and with hypoenergetic feeding (10,11). Recently, we reported that postabsorptive rates of endogenous leucine rate of appearance (R_a) (index of protein catabolism) are increased in obese compared with lean women (12). Further, it is well known that when the portal vein glucagon/insulin relationship favors GNG, its rate can be controlled by the supply of substrate from the periphery (9,13). Taken together, these data led us to hypothesize that elevated protein catabolism in obesity contributes to higher GNG via increased availability of gluconeogenic amino acids.

To study the roles of insulin on glucose and protein metabolism in obesity, postabsorptive GNG was measured in lean and obese subjects after a 17-h fast by the deuterated water method (14,15), using ^2H nuclear magnetic resonance (NMR) (16,17). EGP, protein turnover, and insulin sensitivity were assessed by tracer and clamp methods. We found a strong correlation between insulin resistance, rates of protein catabolism, and the rate of GNG from phosphoenol pyruvate (PEP; GNG_{PEP}) but not from GNG from glycerol. These data suggest that increased protein catabolism and the resulting influx of amino acids to the liver result in increased GNG in obese subjects. Results have been presented in part in abstract form (18).

RESEARCH DESIGN AND METHODS

Nineteen lean and 16 obese men and women were recruited and screened by medical history, physical examination, and laboratory investigation, as previously detailed (19). They were admitted to the McGill University Health Centre, Royal Victoria Hospital's Clinical Investigation Unit after giving written informed consent. The human ethics review committee of the hospital approved the protocol. During the 7 days before the clamp experiment, subjects took no medications and received an individualized formula-based isoenergetic diet, according to resting metabolic rate measured by indirect calorimetry (Deltatrac; Sensor Medics, Yorba Linda, CA), multiplied by a

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EGP, endogenous glucose production; FFA, free fatty acid; FFM, fat-free mass; GLY, glycogenolysis; GNG, gluconeogenesis; NMR, nuclear magnetic resonance; PEP, phosphoenolpyruvate; REE, resting energy expenditure.

physical activity factor of 1.7 for the lean and 1.6 for the obese subjects. Details of the diet and dietary protocol have been previously described (20). Nitrogen balance studies were conducted during the last 3 days of the adaptation diet, as previously described (10). Body composition was assessed by bioelectrical impedance analysis using the RJL-101A Systems (Detroit, MI) instrument and using equations validated for lean (21) and obese (22) men and women. Women with regular menstrual cycles were studied during the follicular phase.

Deuterated water method. The protocol followed was as previously reported (14,15). On day 6, fasting was started at 1800. At 2300 (5 h into fast), subjects drank 3.5 g/kg of body water of ^2H_2O (99.9% ^2H_2O; CDN Isotopes, Pointe-Claire, QC, Canada). Body water was calculated as 0.73 × fat-free mass (FFM), estimated by bioelectrical impedance analysis. After an initial dose of ^2H_2O, subjects were allowed to drink water enriched at 0.35% of ^2H_2O ad libitum. Twenty milliliters of venous blood were drawn for deuterium enrichment of plasma glucose at 1100 of day 7 (17-h fast) before the start of the clamp.

Hyperinsulinemic-euglycemic-isoaminoacidemic clamp protocol. The hyperinsulinemic clamp experiment was performed according to the detailed procedure recently published (20), with target plasma glucose levels at 5.5 mmol/l and maintenance of individual subjects' postabsorptive plasma branched-chain amino acid concentrations. Briefly, glucose turnover was studied using a primed (22 μCi [814 kBq]), continuous infusion (0.22 μCi/min [8.14 kBq/min]) of [3-^{3}H]-glucose, started 180 min before insulin and maintained for the duration of the clamp. At the same time, a primed (0.5 mg/kg), constant infusion of [1-^{13}C] leucine was started at 0.008 mg · kg^{-1} · min^{-1}, to study leucine kinetics (23), after an oral bolus of 0.1 mg/kg of $NaH^{13}CO_3$. A primed infusion of biosynthetic regular human insulin (Humulin R; Eli Lilly Canada, Toronto, ON, Canada) was started at 0 min and maintained at a rate of 40 mU · m^{-2} · min^{-1} for at least 210 min. At 4 min, sterile 20% (wt/vol) potato starch–derived glucose (dextrose anhydrous; Avebe, Foxhol, the Netherlands) in water with added [3-^{3}H]-glucose (the "hot GINF" method [24]) was infused at variable rates to reach and maintain euglycemia. Baseline concentrations of plasma individual amino acids were maintained with an infusion of a 10% amino acid solution (10% TrophAmine without electrolytes; B. Braun Medical, Irvine, CA) by feedback adjustments of infusion rates based on plasma branched-chain amino acid concentrations measured every 5 min from an enzymatic fluorometric assay (20).

Blood samples were collected every 10 min for 40 min before the insulin infusion then every 30 min until the last 40 min, at which time they were again drawn at 10-min intervals. Indirect calorimetry was performed for 20 min before and during the last 30 min of the insulin infusion. Glucose turnover was calculated as specified in (24,25). Expired air samples were collected into a breath collection balloon and transferred to 10-ml Vacutainer tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Leucine kinetics were calculated according to the stochastic model (23), using plasma α-keto isocaproic acid as an index of the precursor pool enrichment (reciprocal model). Correction for dilution in the background enrichment of expired $^{13}CO_2$ and recovery of ^{13}C from the bicarbonate pool was made according to factors determined previously in lean and obese subjects under our experimental conditions (12,20).

Measurements of sources of plasma glucose by ^{2}H NMR of monoacetone glucose. Glucose was isolated by high-performance liquid chromatography from a deionized solution from $Ba(OH)_2$ and $ZnSO_4$ deproteinized plasma, as previously described (15). The effluent was lyophilized and kept sealed at room temperature until analyzed for ^{2}H enrichment in glucose by ^{2}H NMR. This analytical method is not affected by the [1-^{13}C] leucine infused (26) and has been validated against other methods (27,28). Purified glucose was converted to the 1,2-diisopropylidene glucofuranose derivative (monoacetone glucose) as previously described (16,17). Deuterium NMR spectra of monoacetone glucose were collected using a 14.1-T Varian INOVA spectrometer and 3-mm broadband probe, tuned to 92 MHz as previously described (17,27). A 90° pulse was applied, and the signal was acquired over 1 s (sweep width = 1,000 Hz) with no further delay. Waltz-16 decoupling was used to remove proton-deuterium J-coupling. Deuterium spectra were collected in blocks of 256 acquisitions without lock and later synchronized. Total acquisition time was 8–12 h per sample. ^{2}H relative enrichments were determined from the peak areas of each position using the curve-fitting routine supplied with NUTS PC-based NMR spectral analysis program (Acorn NMR, Freemont, CA).

During in vivo synthesis, the protons on the backbone of glucose become enriched with deuterium according to the level of body water enrichment and the activity of the pathways responsible for the exchange (14,15). While all seven proton positions of glucose become enriched during in vivo metabolism, enrichment at the H2, H5, and $H6_S$ are diagnostic of fluxes through GLY and GNG. The fractions of GLY, GNG from glycerol ($GNG_{Glycerol}$) and GNG from the trichloroacetic acid cycle–derived PEP (GNG_{PEP}) were determined from the following relationships (29): GLY = 1 − (H5/H2), $GNG_{Glycerol}$ = (H5 − $H6_s$)/H2 and GNG_{PEP} = ($H6_s$/H2). The corresponding fluxes (GLY flux and GNG_{PEP} flux) were calculated by multiplying these relative contributions by EGP.

Other assays. Plasma glucose was measured by the glucose oxidase method (GM7 Micro-Stat; Analox Instruments USA, Lunenberg, MA). Assays for immunoreactive insulin and glucagon and glucose specific activity were as described (24,30). Plasma amino acids of interest were determined by ion-exchange high-performance liquid chromatography with postcolumn ninhydrin detection (31) using a Beckman HPLC System (Beckman Coulter, Fullerton, CA). Plasma free fatty acids (FFAs) were determined using the NEFAC test kit (Wako Chemicals USA, Richmond, VA). Plasma cortisol was measured by an automated method at the Biochemical Laboratory of the Royal Victoria Hospital. The [^{13}C] enrichment of plasma α-keto isocaproic acid was analyzed by gas chromatography–mass spectrometry (GCMS 5988A; Hewlett-Packard, Palo Alto, CA) after derivatization with *N*-methyl-*N*-(tert-butyldimethylsilyl) trifluoroacetamide (Regis Technologies, Morton Grove, IL) to yield a TBDMS derivative of hydroxyisocaproic acid. Expired air was analyzed for $^{13}CO_2$ enrichment by isotope ratio mass spectrometry on a Micromass 903D (Vacuum Generators, Winsforce, U.K.).

Statistical analyses. Results are presented as means ± SE. Since there were no sex differences in the primary outcomes (EGP and the percent contribution from GNG, GLY, and glycerol), data of men and women were combined. The effect of obesity was assessed by ANOVA, including FFM as a covariate (ANCOVA) when it was found to have a significant predictive value on the dependent variable from prior regression analysis. Pearson's coefficient was used to correlate GNG with other parameters, and when required, partial correlations were performed to control for FFM. Significance level was set at $P < 0.05$. The analyses were performed with SPSS 11.0 for Windows (SPSS, Chicago, IL).

RESULTS

Mean age was greater in the obese subjects (Table 1). The obese group showed the typical indexes of adiposity with greater weight, BMI, percent body fat, waist and hip circumferences, and waist-to-hip ratio. Their FFM was higher than in the lean group. Energy intake was higher in the obese subjects as total per day but not when adjusted for FFM, whereas protein intake was higher as a total per day and when adjusted for FFM. Resting energy expenditure (REE) was higher in the obese subjects, even after adjustment for FFM. The groups were in nitrogen equilibrium (data not presented). Obese subjects had higher fasting insulin and lower glucagon-to-insulin ratio. Serum cortisol and FFAs were not different between groups, but higher FFA concentrations were found in women (not shown).

In the postabsorptive state (Table 2), the fractional contribution of GNG_{PEP} was higher in obese compared with lean subjects, while GLY was proportionally lower (by a total of 10% in both cases). There were no significant differences in the contribution by $GNG_{Glycerol}$ in percent or in flux (not shown). In obese subjects, the total GNG and GNG_{PEP} fluxes were also significantly higher, and the GLY flux less, but only when adjusted for FFM. Plasma concentration of glucose was similar between groups. EGP and glucose rates of disposal (R_d) were not different between lean and obese subjects, whether expressed as a total per body or adjusted for FFM.

Postabsorptive endogenous leucine R_a (index of protein catabolism) and nonoxidative leucine R_d (index of protein synthesis) rates were higher in obese than in lean subjects, whereas oxidation and net leucine balance did not differ (Table 3). Plasma leucine and branched-chain amino acid concentrations were higher in obese than in lean subjects, while those of alanine and glutamine were not different. The sum of the gluconeogenic amino acids was not different between groups.

Several correlations were found between GNG_{PEP} and obesity and with its associated body composition and

TABLE 1
Subject characteristics

	Lean	Obese
n (men/women)	10/9	7/9
Age (years)	26 ± 1	40 ± 3*
Height (cm)	170 ± 3	169 ± 3
Weight (kg)	63 ± 2	99 ± 4*
BMI (kg/m^2)	21.7 ± 0.4	34.8 ± 1.3*
FFM (kg)	50.5 ± 2.3	58.1 ± 2.7†
Body fat (%)	19.7 ± 2.0	41.0 ± 2.0*
Waist circumference (cm)	75 ± 2	108 ± 3*
Hip circumference (cm)	93 ± 1	117 ± 3*
Waist-to-hip ratio	0.80 ± 0.02	0.93 ± 0.02*
Energy intake (adjusted for FFM) (kcal/day)	2,453 ± 90 (2,586 ± 37)	2,801 ± 118 (2,643 ± 41)†
Protein intake (adjusted for FFM) (g/day)	89 ± 4 (94 ± 1)	106 ± 4 (100 ± 1)†
REE (adjusted for FFM) (kcal/day)	1,512 ± 49 (1,585 ± 28)	1,800 ± 71 (1,714 ± 31)†
Insulin (pmol/l)	68 ± 4	113 ± 12*
Glucagon (pmol/l)	19 ± 1	23 ± 2
Glucagon/insulin	0.29 ± 0.02	0.22 ± 0.02†
Cortisol (μmol/l)	519 ± 42	493 ± 44
FFAs (μmol/l)	602 ± 58	647 ± 40

Data are means± SE. *$P = 0.001$, †$P < 0.05$, obese vs. lean group.

metabolic alterations. The GNG_{PEP} contribution to glucose production was positively related to percent body fat ($r = 0.518$, $P = 0.001$; Fig. 1*A*) and to BMI ($r = 0.467$, $P = 0.005$). Furthermore, the gluconeogenic flux also correlated positively with percent body fat, BMI, and waist and hip circumferences (partial r controlled for FFM from 0.464 to 0.494, $P < 0.01$). GNG_{PEP} correlated positively with serum FFAs ($r = 0.367$, $P = 0.030$; Fig. 1*B*) and with plasma insulin ($r = 0.448$, $P = 0.007$) but not with plasma alanine and glutamine concentrations. Inversely, %GLY and GLY flux were negatively related to indexes of adiposity, serum FFAs, and fasting insulin. GNG_{PEP} flux, but not GLY flux, was positively correlated with REE ($r = 0.498$, $P = 0.002$; partial correlation controlled for FFM: $r = 0.419$, $P = 0.014$; Fig. 1*C*). Postabsorptive protein catabolic (Fig. 2) and synthetic rates correlated positively with the GNG_{PEP} flux ($r = 0.476$ and 0.505, $P < 0.005$) and also when controlled for FFM (partial $r = 0.372$ and 0.407, respectively, $P \leq 0.03$). No correlations were found between $\%GNG_{Glycerol}$ or $GNG_{Glycerol}$ flux and all the other variables tested.

The obese group demonstrated insulin resistance of both glucose and protein metabolism as determined by the hyperinsulinemic-euglycemic-isoaminoacidemic clamp (Table 4). These results are consistent with our previous studies in obese women (12) and were not dependent on sex. In response to the same insulin infusion per surface area during the clamp, plasma insulin concentrations increased more in the obese than in the lean subjects (increase of 647 ± 20 vs. 512 ± 52 pmol/l, $P = 0.016$). Endogenous production of glucose was less suppressed in the obese subjects, and glucose R_d was less increased than in the lean subjects. Maximal suppression of serum FFAs was delayed in obese subjects. Protein catabolism was equally suppressed in both groups, but synthesis was less stimulated in the obese group. As a result, the change in protein balance (anabolic response) was less in the obese subjects, and the amino acid infusion rates required to maintain baseline levels were less. This correlated with lower glucose infusion rates in obese subjects during hyperinsulinemia.

To evaluate the association between insulin resistance in obesity and GNG, we performed the following correlations and multiple regression analyses. As shown in Fig. 3*A*, a significant negative relationship was found between the GNG_{PEP} and the anabolic response ($r = -0.538$, $P = 0.001$; when controlled for FFM, partial $r = -0.577$, $P < 0.001$). The percent contribution of GNG_{PEP} was also negatively associated with the clamp glucose R_d (Fig. 3*B*: $r = -0.466$, $P = 0.005$; when controlled for FFM, partial

TABLE 2
Glucose production and utilization, and contribution of GNG glycerol, and GLY to glucose production in lean and obese subjects

	Lean	Obese
$\%GNG_{PEP}$ ($H6_S/H2 \times 100$)	41 ± 2	52 ± 2*
$\%GNG_{Glycerol}$ [$(H5 - H6_S)/H2 \times 100$]	10 ± 1	9 ± 2
%GLY [$(1 - H5/H2) \times 100$]	49 ± 2	39 ± 3*
Total GNG flux (adjusted for FFM) (μmol/min, in hexose units)	357 ± 13 (366 ± 19)	469 ± 27 (459 ± 21)*
GNG_{PEP} flux (adjusted for FFM) (μmol/min, in hexose units)	288 ± 16 (294 ± 20)	396 ± 26 (389 ± 22)*
GLY flux (adjusted for FFM) (μmol /min)	354 ± 27 (379 ± 20)	305 ± 26 (276 ± 22)*
EGP (adjusted for FFM) (μmol/min)	711 ± 28 (743 ± 19)	774 ± 34 (735 ± 21)
Glucose R_d (adjusted for FFM) (μmol/min)	715 ± 27 (746 ± 22)	781 ± 35 (743 ± 23)
Plasma glucose (mmol/l)	4.8 ± 0.1	5.0 ± 0.1

Data are means ± SE. GNG_{PEP}: GNG from PEP derived from the trichloroacetic acid cycle (in hexose units). $GNG_{glycerol}$: GNG from glycerol (in hexose units). $\%GNG_{PEP}$, $\%GNG_{glycerol}$, and %GLY to EGP were calculated from the relative enrichments of glucose H2, H5, and $H6_S$ of glucose measured by ^{2}H NMR. *$P < 0.05$, obese vs. lean group.

TABLE 3
Postaborptive leucine kinetics and plasma gluconeogenic amino acids in lean and obese subjects

	Lean	Obese
Endogenous leucine R_a (μmol/min)*	130 ± 3	149 ± 3†
Leucine oxidation (μmol/min)	29 ± 1	31 ± 1
Nonoxidative leucine R_d (μmol/min)	102 ± 2	118 ± 3†
Net leucine balance (μmol/min)	−29 ± 1	−31 ± 1
Branched-chain amino acids (μmol/l)	379 ± 11	423 ± 20‡
Alanine (μmol/l)	324 ± 15	345 ± 15
Glutamine (μmol/l)	520 ± 22	481 ± 13
Total GNG amino acids (μmol/l)	1,372 ± 139	1,300 ± 86

Data are means± SE. *Leucine kinetics are adjusted for FFM (see STATISTICAL ANALYSES). GNG amino acids: sum of gluconeogenic amino acids (alanine, glutamine, glutamic acid, glycine, threonine, and serine). †$P = 0.001$, ‡$P < 0.05$, obese vs. lean group.

$r = -0.444$, $P = 0.009$). From stepwise multiple regression analysis, GNG_{PEP} correlated most with percent body fat ($R^2 = 0.270$, $P = 0.002$), while 35% of the variance of the GNG_{PEP} flux was explained by body weight.

DISCUSSION

This study shows that the fractional and absolute contribution of GNG to glucose production is higher in obesity and is associated with increased rates of protein turnover and insulin resistance of glucose, lipid, and protein metabolism. Our methodology cannot distinguish between hepatic and renal contributions to EGP, though the liver is likely preponderant in our protocol of an overnight fast. The higher contribution of total GNG to glucose production was entirely due to the higher fraction of GNG_{PEP}, as $GNG_{Glycerol}$ was not different between lean and obese subjects. EGP did not differ between groups because the fractional contribution from GLY was correspondingly decreased in obese subjects, suggesting relatively intact hepatic autoregulation, considering their hyperinsulinemia and that the increased contribution of GNG_{PEP} was unlikely to have resulted from increased Cori cycle activity. The fact that $GNG_{Glycerol}$ was not different between obese and lean subjects is somewhat surprising, since obesity, in general, is associated with higher rates of lipolysis (32), which should contribute to excess glycerol as a substrate for hepatic GNG. Among subjects with HIV-associated lipoatrophy, triglyceride turnover is elevated, and these subjects had significantly higher rates of $GNG_{Glycerol}$ than control subjects (33). Absolute glycerol turnover is elevated in obese subjects but not when expressed per fat mass (32). Whereas a number of studies have demonstrated elevated GNG in obesity, GNG from glycerol (reported as $GNG_{Glycerol}$) has not been distinguished from GNG from lactate and amino acids (reported as GNG_{PEP}) as we have done here. Our data suggest that lipolysis derived glycerol contributes the same percentage and flux as in lean subjects, to the elevated GNG found in obese nondiabetic subjects.

The method used here to distinguish between GNG_{PEP} and $GNG_{Glycerol}$ is based on the difference between the deuterium enrichment at H5 and $H6_S$. Enrichment at H5 occurs at the level of the triosephosphate isomerase reaction, while $H6_S$ enrichment occurs at the level of fumarase in the trichloroacetic acid cycle. Thus, an accurate measurement of $GNG_{Glycerol}$ assumes that exchange at the level of oxaloacetic acid and fumarate is complete, as has been previously proposed (34). However, Landau et al. (15) suggested that H6 enrichment is an underestimate of PEP-derived GNG because of exchange with body water that was ~20% incomplete. But in those experiments, $H6_R$

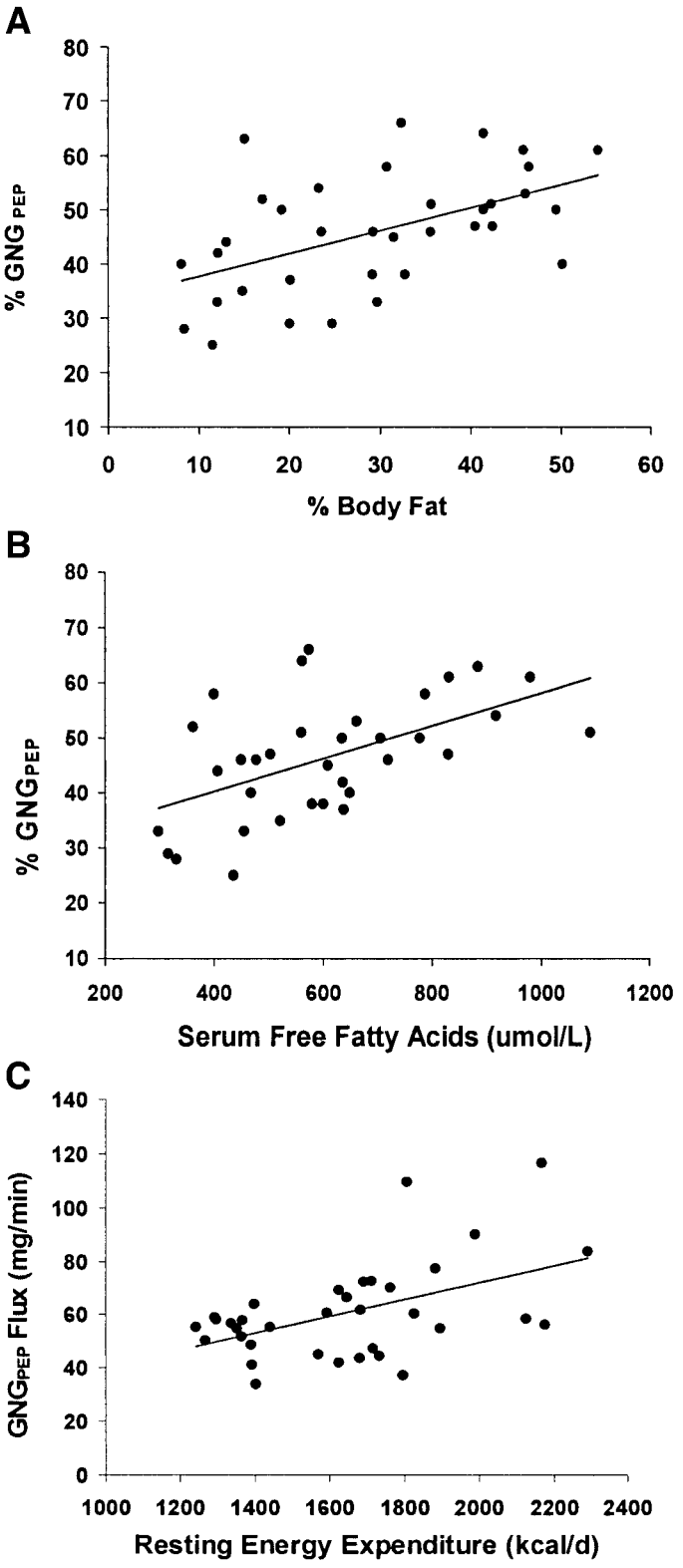

FIG. 1. Correlations between percent contribution of GNG_{PEP} to postabsorptive glucose production and percent body fat: $r = 0.518$, $P = 0.001$ ($n = 35$) (*A*), serum FFAs: $r = 0.546$, $P = 0.001$ ($n = 34$) (*B*), and GNG_{PEP} flux and REE: $r = 0.498$, $P = 0.002$ (*C*); partial correlation controlled for FFM: $r = 0.419$, $P = 0.014$.

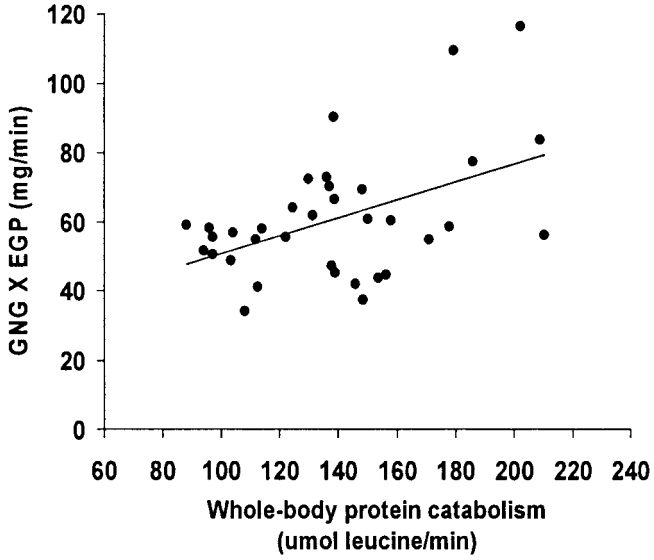

FIG. 2. Correlation between gluconeogenic flux via PEP and postabsorptive whole-body protein catabolism: $r = 0.476$, $P = 0.004$ ($n = 35$); partial correlation controlled for FFM: $r = 0.372$, $P = 0.030$.

and $H6_S$ were not distinguished from each other, so that incomplete exchange between pyruvate and alanine was a potential problem. We do not believe this is a problem with the conversion of oxaloacetic acid to fumarate, which specifically labels $H6_S$ because that exchange is many times faster than GNG from PEP (34). To the extent that exchange is incomplete, GNG_{PEP} would be slightly underestimated, while $GNG_{Glycerol}$ would be overestimated to exactly the same extent. Nevertheless, there is no reason to suspect that the extent of exchange (complete or not) is different between lean and obese subjects so our comparison of $GNG_{Glycerol}$ between the two under identical conditions should be valid.

Elevated GNG in human obesity (2–4) has been correlated with percent body fat and visceral fat (3), increased serum levels of FFAs (3,35), and we found BMI, waist and hip circumferences, waist-to-hip ratio, and fasting insulin concentrations to be related as well. The correlation of GNG_{PEP} with REE is consistent with the higher energy cost of increased protein turnover and of GNG. In type 2 diabetes, elevated GNG is associated with high fasting glucose (1–3,8,36) and elevated glucagon-to-insulin ratio (37). It has also been shown that in the presence of type 2 diabetes obesity had an independent and additive effect in further increasing the %GNG (3).

TABLE 4
Changes in leucine and glucose kinetics in lean and obese subjects in response to the hyperinsulinemic-euglycemic-isoaminoacidemic clamp

	Lean	Obese
Endogenous leucine R_a (μmol/min)*	−24 ± 2	−23 ± 3
Leucine oxidation (μmol/min)	5 ± 1	9 ± 2‡
Nonoxidative leucine R_d (μmol/min)	19 ± 2	6 ± 3†
Net leucine balance (μmol/min)	43 ± 2	28 ± 2†
Amino acid infusion (mg/min)	44 ± 1	35 ± 1†
EGP (mg/min)	−137 ± 5	−120 ± 5‡
Glucose R_d (mg/min)	350 ± 20	179 ± 22†
Glucose infusion (mg/min)	491 ± 23	300 ± 25†

Data are means± SE. *All data are adjusted for FFM (see statistical analyses). †$P = 0.001$; ‡$P < 0.05$, obese vs. lean group.

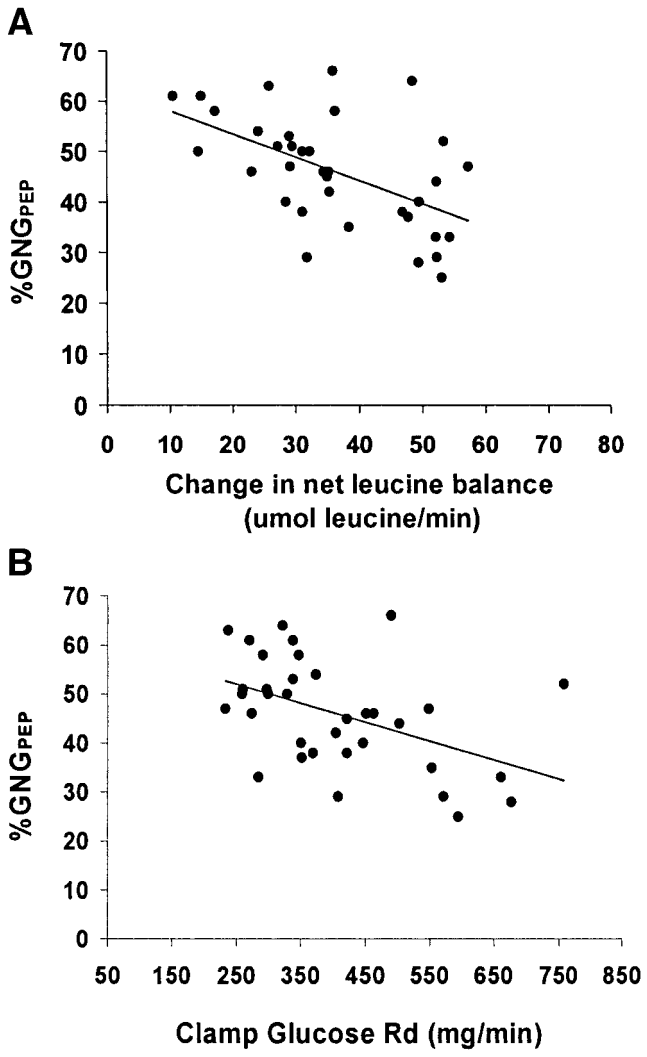

FIG. 3. Correlations between percent contribution of GNG_{PEP} to postabsorptive glucose production and the change in whole-body net leucine balance during the hyperinsulinemic clamp (anabolic response to insulin): $r = -0.538$, $P = 0.001$ ($n = 35$) (*A*), partial correlation controlled for FFM: $r = -0.577$, $P < 0.030$, and clamp glucose rate of disposal (R_d): $r = -0.468$, $P = 0.005$ ($n = 35$) (*B*); partial correlation controlled for FFM: $r = -0.444$, $P = 0.009$.

Our group of obese subjects presented the typical metabolic alterations associated with insulin resistance: higher fasting plasma insulin concentrations, blunted suppression of glucose R_a and FFAs, and lesser glucose R_d during the hyperinsulinemic clamp compared with the lean group. We also observed insulin resistance of whole-body protein turnover evidenced by lower amino acid infusion rates to maintain postaborptive plasma levels during a hyperinsulinemic clamp and a lesser stimulation of protein synthesis by insulin. These data are consistent with our recent findings in obese women (12), demonstrating that the effect is not sex specific.

The positive correlation of GNG_{PEP} flux with postabsorptive protein catabolic rates is a new finding in human obesity, relating adiposity to abnormalities of protein metabolism. We postulate that increased protein catabolism increases gluconeogenic amino acid availability and thus independently augments GNG in the presence of the appropriate hormonal milieu. To our knowledge, such a

direct association has not been previously reported in humans. Nonetheless, studies in which amino acids were provided orally or intravenously, such that plasma levels were elevated, have reported either higher glucose production and/or increased GNG, supporting a direct gluconeogenic effect of amino acids (13,38). Provision of exogenous alanine to prolonged-fasted obese people increased glucose production without glucoregulatory hormone alterations (39). Postprandial elevations of plasma amino acids with maintenance of fasting levels of insulin and glucagon during a somatostatin clamp increased GNG without any effect on GLY and led to hyperglycemia. When insulin was allowed to rise during the amino acid infusion, glycemia was normal but resulted again from a greater contribution of GNG (96%) and a minute contribution from GLY (38,40). Therefore, greater endogenous gluconeogenic amino acid release from increased protein catabolism may trigger their preferential use over glycogen as a source of glucose in obese subjects. That plasma gluconeogenic amino acid levels were not elevated in parallel with higher catabolism may be due to the correspondingly greater hepatic uptake of these amino acids. This is supported by studies in the conscious dog model showing that net hepatic uptake of glutamine was increased during infusion of a mixture of gluconeogenic amino acids but more so via the portal than peripheral route (40). Furthermore, in obese humans, the fractional splanchnic extraction of the glucose precursors, alanine and lactate, was higher than in lean subjects (41).

Although increased GNG has never been directly associated with increased rates of protein catabolism, elevated amino acid turnover must impact hepatic GNG because of the liver's principal role in amino acid catabolism. The liver is the only organ containing the complete set of urea cycle enzymes, so it is critical in handling amino acid disposal. Upon transfer of the nitrogen from amino acids such as alanine and glutamine to urea, the carbon backbone of the amino acid must be disposed of by anaplerosis into the hepatic trichloroacetic acid cycle followed by conversion to glucose. Thus, nitrogen disposal is inextricably linked to anaplerosis and GNG in the liver. Increased GNG has been found in many catabolic conditions, for example, in type 2 diabetes (1–3) and lung cancer (42) in humans and in muscle unloading (43) and acute uremia (44) in rats. In the latter model, GNG from alanine, glutamine, and serine was increased concomitantly with muscle protein breakdown and normalized with administration of an antiglucocorticoid, suggesting a substrate-driven effect on GNG. Although obesity is not considered a catabolic state, postaborptive whole-body protein turnover rates were higher in our obese than in lean subjects, even when differences in lean tissue mass were taken into account. Therefore, a more rapid turnover resulting from both higher rates of release and uptake by tissues, hence unchanged blood concentrations of GNG amino acids, may increase the amino acid supply to serve as glucose precursors. From the study of selected regional tissues, it has been demonstrated that in the postabsorptive state, skeletal muscle protein breakdown is greater than synthesis, whereas the inverse situation is seen in the splanchnic region (45). The increased muscle catabolism thus provides the amino acids for both increased liver protein synthesis and GNG.

In the present study, EGP was normal in obese compared with lean subjects because even though GNG_{PEP} was elevated, GLY was reduced. Hepatic glycogen stores have been found to be higher in obese humans (4), and GLY was shown to be suppressed even by small elevations in plasma insulin in dogs (46) by regulating hepatic glycogen cycling (47). It may be argued that a higher level of fasting insulin in the obese subjects is responsible for lower contribution of GLY to glucose production and thus higher glycogen stores. If this is the case, it is unclear why GNG is not also reduced, since chronic hyperinsulinemia normally suppresses PEPCK and glucose-6-phosphatase expression and activity (48,49). Perhaps this underscores the fact that both peripheral and hepatic insulin resistance are present in obesity and that the normal regulation of hepatic enzyme activities is resistant to increased fasting insulin in obese subjects. It is possible that despite a certain degree of hepatic insulin resistance, GLY was suppressed through the autoregulatory process as a direct result of increased GNG driven by substrate supply (5). This is supported by unaltered EGP in obesity even in the presence of higher insulin concentrations. Of note is that PEPCK is also stimulated by FFAs (50). Although FFA concentrations were not different between lean and obese subjects in the present study, they were correlated with increased GNG, suggesting a possible mechanism contributing to our results.

In summary, we found that increased contribution of GNG_{PEP} (but not $GNG_{Glycerol}$) to glucose production in obesity is linked to increased protein catabolism and insulin resistance of both glucose and protein metabolism. Hepatic glucose production was normal through a correspondingly lower contribution from GLY. High protein catabolism appears to provide supplemental gluconeogenic amino acids to the liver that trigger their preferential use over glycogen for glucose production.

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