Time course of postprandial hepatic phosphorus metabolites in lean, obese, and type 2 diabetes patients[1,2]

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ABSTRACT

Background: Impaired energy metabolism is a possible mechanism that contributes to insulin resistance and ectopic fat storage.

Objective: We examined whether meal ingestion differently affects hepatic phosphorus metabolites in insulin sensitive and insulin resistant humans.

Design: Young, lean, insulin-sensitive humans (CONs) [mean ± SD body mass index (BMI; in kg/m^2): 23.2 ± 1.5], insulin-resistant, glucose-tolerant, obese humans (OBEs) (BMI: 34.3 ± 1.7), and type 2 diabetes patients (T2Ds) (BMI: 32.0 ± 2.4) were studied (n = 10/group). T2Ds (61 ± 7 y old) were older ($P < 0.001$) than were OBEs (31 ± 7 y old) and CONs (28 ± 3 y old). We quantified hepatic γATP, inorganic phosphate (Pi), and the fat content [hepatocellular lipids (HCLs)] with the use of ^{31}P/^{1}H magnetic resonance spectroscopy before and at 160 and 240 min after a high-caloric mixed meal. In a subset of volunteers, we measured the skeletal muscle oxidative capacity with the use of high-resolution respirometry. Whole-body insulin sensitivity (M value) was assessed with the use of hyperinsulinemic-euglycemic clamps.

Results: OBEs and T2Ds were similarly insulin resistant (M value: 3.5 ± 1.4 vs. 1.9 ± 2.5 mg · kg^{-1} · min^{-1}, respectively; $P = 0.9$) and had 12-fold ($P = 0.01$) and 17-fold ($P = 0.002$) higher HCLs than those of lean persons. Despite comparable fasting hepatic γATP concentrations, the maximum postprandial increase of γATP was 6-fold higher in OBEs (0.7 ± 0.2 mmol/L; $P = 0.03$) but only tended to be higher in T2Ds (0.6 ± 0.2 mmol/L; $P = 0.09$) than in CONs (0.1 ± 0.1 mmol/L). However, in the fasted state, muscle complex I activity was 53% lower ($P = 0.01$) in T2Ds but not in OBEs ($P = 0.15$) than in CONs.

Conclusions: Young, obese, nondiabetic humans exhibit augmented postprandial hepatic energy metabolism, whereas elderly T2Ds have impaired fasting muscle energy metabolism. These findings support the concept of a differential and tissue-specific regulation of energy metabolism, which can occur independent of insulin resistance. This trial was registered at clinicaltrials.gov as NCT01229059. *Am J Clin Nutr* doi: 10.3945/ajcn.115.107599.

INTRODUCTION

Nonalcoholic fatty liver diseases (NAFLDs)[8] have emerged as the most-common causes of chronic liver disease in Western countries (1). NAFLDs start from hepatic steatosis as defined by hepatocellular lipids (HCLs) ≥5.56% (1). Although HCLs are frequently increased in obesity and type 2 diabetes and are tightly related to insulin resistance (2), the exact mechanisms that underlie this relation are still unclear.

Several hypotheses have been raised to explain the pathogenesis of hepatic insulin resistance. Chronic hyperglycemia impairs insulin sensitivity by a mechanism termed glucose toxicity (3). In line with this, blood glucose lowering improves the suppression of endogenous glucose production in type 2 diabetes patients (T2Ds) (4). However, insulin-mediated hepatic glycogen synthesis remains abnormal even in well-controlled T2Ds (5), indicating that other mechanisms also contribute to hepatic insulin resistance. Skeletal muscle insulin

resistance relates to a lower energy use during hyperinsulinemia as shown by an impaired muscle ATP synthesis (6) and oxidative capacity (7). Along with hyperinsulinemia, these effects could shift postprandial energy storage away from muscle glycogen synthesis toward hepatic de novo lipogenesis, thereby increasing HCLs (8). Impaired hepatic lipid oxidation could promote postprandial HCL accumulation and hepatic insulin resistance (9). Indeed, reduced mitochondrial β oxidation (10, 11) and mitochondrial content (12) were associated with increased HCLs in some rodent models. In contrast, obese individuals without NAFLDs have an increased hepatic oxidative capacity in the fasted state, which is lost in obese patients with inflammatory NAFLDs (13). This discrepancy results from the adaptive upregulation of mitochondrial oxidative phosphorylation (OXPHOS) in obesity (13), which enhances the production of reactive oxygen species but causes mitochondrial inefficiency and damage in longstanding T2Ds (14, 15). Mitochondrial adaptation would be particularly important under conditions of increased substrate flow such as meal ingestion. To our knowledge, no data on postprandial energy metabolism are available for human livers.

Thus, this study examined the effects of a single mixed meal on the time course of hepatic energy metabolism with the use of magnetic resonance spectroscopy (MRS) in young, lean, insulin-sensitive humans (CONs), in age-matched insulin-resistant, glucose-tolerant, obese humans (OBEs)], and in elderly T2Ds with comparable degrees of obesity and insulin resistance. We hypothesized that obese, insulin-resistant humans would *1*) respond to a standardized mixed meal with enhanced hepatic energy metabolism but *2*) exhibit lower postabsorptive (fasting) energy metabolism in skeletal muscle.

METHODS

Participants and study design

We included 10 CONs, 10 OBEs, and 10 T2Ds. T2Ds were matched for sex and body fat mass with OBEs (**Table 1**). All participants underwent the recording of a medical history as well as physical and laboratory examinations. After inclusion, subjects received a standardized mixed-meal test (MMT) (visit 1), a hyperinsulinemic-euglycemic clamp test along with a muscle biopsy and the measurement of the oxidative capacity during fasting and the use of indirect calorimetry during fasting and clamp conditions (visit 2) (17), and the measurement of the muscle fat content (visit 3) on separate visits spaced ≥2 wk. For 3 d before each visit, participants refrained from physical activity and alcohol ingestion and adhered to a balanced isocaloric diet. T2Ds were receiving treatment with metformin ($n = 8$), sulfonylurea ($n = 1$), and glucagon-like peptide-1 analoga ($n = 1$) but had never used insulin or thiazolidinediones. TD2s had withdrawn their glucose- and lipid-lowering drugs 3 d before the tests as was done in previous studies by our group and other groups (4, 5, 18, 19). Some volunteers had also participated in other studies of our group [3 OBEs, 2 T2Ds, and 8 CONs (16); 3 OBEs, 5 T2Ds, and 1 CONs (7)], but to our knowledge, no data from the MMT or on hepatic energy metabolism have been reported previously. All participants gave their informed consent to the study protocols, which

TABLE 1
Characteristics of CONs, OBEs, and T2Ds[1]

	CONs	OBEs	T2Ds
n (men/women)	10 (6/4)	10 (6/4)	10 (6/4)
Age, y	28.0 ± 2.5^a	31.0 ± 6.5^a	61.3 ± 7.0
BMI, kg/m^2	$23.2 \pm 1.5^{a,b}$	34.3 ± 1.7^a	32.0 ± 2.4
Waist circumference, cm	$79.9 \pm 6.7^{a,b}$	113.3 ± 7.6	108.8 ± 10.9
Waist:hip ratio	$0.8 \pm 0.1^{a,b}$	1.0 ± 0.1	1.0 ± 0.1
Fat mass, %	$22.1 \pm 5.4^{a,b}$	36.2 ± 5.9	39.6 ± 3.8
Free fatty acids, μmol/L	353 ± 170^a	502 ± 93	571 ± 192
Triglycerides, mg/dL	$65 \pm 2^{a,b}$	124 ± 2	124 ± 1
Glucose, mg/dL	75 ± 5^a	79 ± 9^a	138 ± 46
Hemoglobin A1C, %	5.1 ± 0.3^a	5.3 ± 0.3^a	7.2 ± 1.4
Hemoglobin A1C, mmol/mol	30.7 ± 2.7^a	32.5 ± 3.5^a	51.8 ± 14.1
Insulin, μU/mL	$4.7 \pm 2.0^{a,b}$	13.8 ± 1.5	10.1 ± 1.9
M value, mg · kg^{-1} · min^{-1}	$8.8 \pm 1.4^{a,b}$	3.5 ± 1.4	1.9 ± 2.5
Alanine aminotransferase, U/L	15 ± 1^b	31 ± 2	22 ± 2
Uric acid, mg/dL	4.7 ± 1.1^b	6.5 ± 1.4	6.0 ± 1.2
HCLs, percentage of H$_2$O	$0.6 \pm 1.77^{a,b}$	4.1 ± 3.3	6.2 ± 3.9
Liver volume, mL	$1496 \pm 245^{a,b}$	2299 ± 393	2023 ± 465
Respiratory quotient	0.78 ± 0.03^a	0.84 ± 0.07	0.87 ± 0.06

[1]All normal distributed data are presented as means ± SDs, and all log-normal distributed variables (insulin, triglycerides, *M* value, alanine aminotransferase, and HCLs) are presented as geometric means ± geometric SDs. [a,b]ANOVA with Bonferroni adjustment for multiple comparisons: [a]$P < 0.05$ vs. T2Ds, [b]$P < 0.05$ vs. OBEs. Anthropometric data, laboratory data, HCLs, and the respiratory quotient were measured during the fasting state. Data for *M* value and the respiratory quotient of 6 OBEs, 7 T2Ds, and 9 CONs were published previously (7, 16). CON, young, lean, insulin-sensitive human; HCL, hepatocellular lipid; *M* value, whole-body insulin sensitivity; OBE, insulin-resistant, glucose-tolerant, obese human; T2D, type 2 diabetes patient.

were approved by the Ethics Board of Heinrich-Heine University Düsseldorf (clinicaltrials.gov; NCT01229059).

Experimental protocols

Participants arrived at the Clinical Research Center after an overnight fast. An intravenous catheter was placed for blood sampling, and ^{31}P/^{1}H MRS was performed to measure absolute concentrations of liver phosphorus metabolites and HCLs before and at 160 and 240 min after ingestion of a high-caloric liquid mixed meal [652 kcal, 55% carbohydrates (71.9% oligosaccharides/polysaccharides, 15.7% saccharose, 2.4% glucose, and <1.2% lactose), 15% protein, and 35% fat] (5). Blood samples were obtained before and 15, 30, 60, 90, 120, 180, and 260 min after the meal to determine blood glucose, hormones, free fatty acids (FFAs), and triglycerides.

Analytic methods

Plasma glucose was measured with the use of the glucose oxidase method (EKF biosen C-Line glucose analyzer; EKF Diagnostic GmbH) (20). FFAs were assayed microfluorimetrically (Wako Chem USA Inc.). In vitro lipolysis was prevented by collecting blood into orlistat-containing vials (21). Triglycerides and liver enzymes were measured enzymatically on a Hitachi analyzer (Roche Diagnostics). Insulin and

C-peptide were determined with the use of a double-antibody radioimmunoassay (22).

MRS

All measurements were performed on a clinical 3 Tesla whole-body magnet (3T Philips X-series Achieva; Philips Healthcare) after appropriate scout images to localize proper voxels of interest (VOIs).

Liver ^{31}P MRS

Localization images were taken with the build-in body ^1H coil, and ^{31}P spectra were obtained with a 14-cm circular ^{31}P surface coil (Philips Healthcare). All methods for the acquisition and processing of ^{31}P liver spectra underwent an extensive validation (23). Three-dimensional localized liver spectra were obtained with the use of the image-selected in vivo spectroscopy sequence with an adiabatic pulse for excitation [VOI: $6 \times 5 \times 6$ cm^3; repetition time (TR): 6 s; signal averages: $n = 128$]. All spectra were fitted with the use of a Java-based version of the magnetic resonance (MR) user interface (EC Human Capital and Mobility Networks). Measured concentrations of phosphorus metabolites were corrected for the volume captured by lipid droplets within hepatocytes (24).

Liver ^1H MRS

To accurately assess the volume occupied by fat in the liver, sets of non–water-suppressed and water-suppressed ^1H MR spectra were acquired with the use of the stimulated echo acquisition mode. The variables were as follows: TR, echo time, and mixing time (4000, 10, and 13 ms, respectively); signal averages ($n = 32$); and VOI ($3 \times 3 \times 2$ cm^3), as previously described (23). Water (4.7 ppm) and fat (1.3-, 0.9-, and 2.1-ppm peaks) were fitted with the use of the NUTS program 2D Professional Version (Acorn NMR Inc.). Data from localized ^1H MRS was analyzed, and absolute concentrations were expressed as percentages of HCLs relative to the water content (25). The liver volume was calculated from axial images with the use of manual segmentation software available in the MR console.

Muscle ^1H MRS

The left calf was positioned with FLEX-S coils (Philips Healthcare). A single-voxel point-resolved spectroscopy sequence (TR and echo time: 2000 and 32 ms, respectively) was used with a voxel size of 2 cm placed within the soleus muscle. The processing of MR spectra was performed with the use of a Java-based version of the MR user interface. All peak areas were obtained with the use of a consistent fitting routine. For the quantification of intramyocellular lipids, the signal from the middle-chain methylene groups (-CH2- at 1.3 ppm) was evaluated relative to water signals in the same voxel (26).

High-resolution respirometry of skeletal muscle

Muscle biopsies were taken from the vastus lateralis muscle under local anesthesia with 2% lidocaine before starting the hyperinsulinemic-euglycemic clamp (16). The isolation and permeabilization of fibers were performed as described (16). The mitochondrial oxidative capacity was measured in permeabilized fibers with the use of a 2-chamber oxygraph (OROBOROS Instruments) (7). Oxygen consumption was corrected for muscle wet mass (2–4 mg) and given as oxygen flux expressed as pmol · g^{-1} · s^{-1}. Defined respiratory states were analyzed in the presence of malate (2.0 mmol/L), ADP (1.0 mmol/L; state 3 complex I), glutamate (10.0 mmol/L), and succinate (10.0 mmol/L; state 3 complexes I and II) with or without pyruvate (5.0 mmol/L). Cytochrome c (10 μmol/L) was added to test the mitochondrial membrane integrity followed by incremental titration steps of 1.0 μL carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (0.1 mmol/L) until the maximal uncoupled respiration (state u) was reached.

Indirect calorimetry

Respiratory quotients were assessed with the use of indirect calorimetry performed in the canopy mode during fasting and steady-state clamp conditions with a postcalorimetrical individual calibration control evaluation to minimize data variability (17).

Calculations and statistics

The AUC was calculated with the use of the trapezoidal method. Nonnormally distributed variables [insulin, triglycerides, whole-body insulin sensitivity (M value), alanine aminotransferase (ALT), and HCLs] were log transformed. Data are presented as means $\pm$ SEMs if not otherwise stated. Comparisons between groups were performed with the use of an ANOVA with Bonferroni adjustment for multiple comparisons. For the MMT, group means at every time point were determined with the use of a maximum likelihood approach as implemented in PROC MIXED procedure (SAS 9.3; SAS Institute Inc.). This method corresponds to a generalized 2-factor ANOVA model that takes into account the dependency between observations at different time points on specific persons.

Because the differences between time curves for glucose, FFAs, triglycerides, and insulin between groups likely differently affect energy metabolism, we specifically analyzed the differences between group means at every time point and differences between time points within each group. Thus, we chose the Bonferroni-based Dunn test (27) because usual omnibus tests cannot be used to address these specific questions (28). This multiple test procedure controls the family-wise error rate in the strong sense at an α level of 5%; which means that the probability of even one type I error is set at the 5% level.

Pearson correlations and corresponding P values that were based on the entire sample were calculated. Partial Pearson correlations adjusted for group effects were derived to examine the role of group membership as a confounding factor. All analyses were performed with SPSS 20.0 software (SPSS Inc.) or SAS 9.3 software as appropriate.

RESULTS

Participant characteristics

Both OBEs and T2Ds had greater BMI, waist circumference, waist:hip ratio, and body fat mass than those of CONs, whereas body fat mass was similar between OBEs and T2Ds (Table 1, **Supplemental Table 1**). Compared with OBEs and CONs, T2Ds were older and hyperglycemic (Table 1, Supplemental

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Table 1). Serum ALT concentrations were higher in OBEs than in CONs but were within the normal range except for 2 participants with mild elevation (51 and 56 U/L) (Table 1, Supplemental Table 1). Uric acid concentrations were higher in OBEs and tended to be higher in T2Ds than in CONs ($P = 0.09$) (Table 1, Supplemental Table 1).

MMT

In T2Ds, the $AUC_{0-260 \, min}$ for plasma glucose was 45% and 42% higher after the MMT than in OBEs and CONs (**Figure 1**A, **Table 2**). Fasting plasma FFAs were 62% higher in T2Ds than in CONs, but the significance of differences was lost after correction for multiple comparisons ($P = 0.12$) (Table 1, Supplemental Table 1). For the MMT, plasma FFAs decreased in all groups with CONs showing the fastest suppression within 120 min ($P < 0.001$) (Figure 1B). The $AUC_{0-260 \, min}$ for FFAs was 50% greater in T2Ds and OBEs (Figure 1B, Table 2) than in CONs. Fasting triglycerides were significant higher in the obese and TD2s, but the significance of differences was lost after correction for multiple comparisons (Table 1, Supplemental Table 1). Postprandial serum triglycerides did not differ between groups after Bonferroni adjustment (Figure 1C, Table 2). Only at 260 min were triglycerides higher in T2Ds than in CONs (Figure 1C). Fasting plasma insulin was 2.4- and 3-fold higher in T2Ds and OBEs than in CONs. After the MMT, insulin release was 2.6-fold higher in OBEs (but not in T2Ds) than in CONs (Figure 1D, Table 2).

Fasting hepatic γATP concentrations were similar in all groups (**Table 3**). At 240 min after the MMT, hepatic γATP had increased by 21% in T2Ds ($P = 0.03$) and by 27% in OBEs ($P = 0.009$) and did not change in CONs (Table 3), but significances of differences were lost after Bonferroni adjustment. The postprandial maximum increase of γATP was 6-fold higher in OBEs ($P = 0.03$) and tended to be higher in T2Ds ($P = 0.09$) than in CONs (**Figure 2**A). Fasting hepatic inorganic phosphate (Pi) was 41% and 32% lower in OBEs and T2Ds, respectively, than in CONs (Table 3), which remained significant only in OBEs after Bonferroni adjustment ($P = 0.02$). The maximum increase of Pi did not differ between groups (Figure 2B). Consequently, the fasting γATP:Pi ratio was 55% greater in OBEs and 38% greater in T2Ds than in CONs (1.6 ± 0.2 and 1.5 ± 0.1 vs. 1.1 ± 0.1, respectively; $P = 0.01$ and $P = 0.04$, respectively), but significances of differences were lost after Bonferroni adjustment ($P = 0.14$ and $P = 0.7$). The γATP:Pi ratio did not change after the MMT in any group.

During fasting, HCLs were 17- and 12-fold higher in T2Ds and OBEs than in CONs (Table 1, Supplemental Table 1). Hepatic steatosis was present in 7 T2Ds and 4 OBEs. The liver volume was higher in T2Ds and OBEs than in CONs (Table 1, Supplemental Table 1). The maximum postprandial increase in HCLs did not differ between groups (T2Ds: $0.67 \pm 0.38\%$; OBEs: $0.95 \pm 0.44\%$; CONs: $0.03 \pm 0.08\%$; $P = 0.16$).

M value and muscle energy metabolism

T2Ds and OBEs had 73% and 61% lower whole-body insulin sensitivity than that of CONs (Table 1, Supplemental Table 1). Fasting respiratory quotients were higher in T2Ds than in CONs (Table 1, Supplemental Table 1). Skeletal muscle state 3 respiration
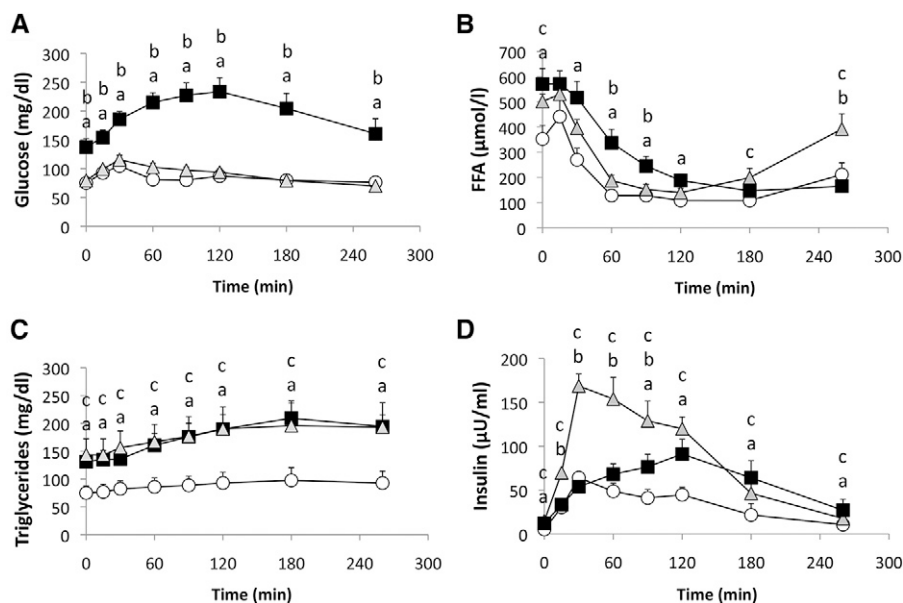

FIGURE 1 Mean ± SEM plasma concentrations of glucose (A), FFA (B), triglycerides (C), and insulin (D) during ingestion of a mixed meal in CONs (white circles; $n = 10$), OBEs (gray triangles; $n = 10$), and T2Ds (black squares; $n = 10$). [a,b,c]Generalized 2-factor ANOVA without Bonferroni adjustment: [a]$P < 0.05$ T2D vs. CONs, [b]$P < 0.05$ OBE vs. T2D, [c]$P < 0.05$ OBE vs. CONs. Differences for glucose at all time points between OBEs, T2Ds, and CONs remained significant after multiple comparisons. Significant differences for FFA were lost after a multiple comparison at all time points apart from higher FFA in T2Ds than in CONs at 30 min ($P = 0.047$) and at 60 min ($P = 0.006$) after the MMT and higher FFA in OBEs than in T2D at 260 min after the MMT ($P = 0.04$). Significant differences for triglycerides were lost at all time points after a multiple comparison apart from higher triglycerides in T2Ds than in CONs at 260 min ($P = 0.04$). Significant differences for insulin were lost at all time points after a multiple comparison apart from higher insulin in OBEs than in CONs at fasting ($P = 0.004$) and at 60 min ($P = 0.005$), 90 min, ($P = 0.001$), and 120 min ($P = 0.01$) after the MMT and from higher insulin in T2Ds than in CONs at 180 min ($P = 0.01$) and 260 min ($P = 0.002$). CON, young, lean, insulin-sensitive human; FFA, free fatty acid; MMT, mixed-meal test; OBE, insulin-resistant, glucose-tolerant, obese human; T2D, type 2 diabetes patient.

TABLE 2
$AUC_{0–260\ min}$ of glucose, FFAs, triglycerides, and insulin after mixed-meal ingestion by CONs ($n = 10$; for insulin, $n = 8$), OBEs ($n = 10$), and T2Ds ($n = 10$; for insulin, $n = 9$)[1]

$AUC_{0–260\ min}$	CONs	OBEs	T2Ds
Glucose, mg/dL × 260 min	21,707 ± 898[a,b]	23,265 ± 908	52,048 ± 5500
FFAs, μmol/L × 260 min	43,869 ± 3629[a,b]	66,668 ± 6235	67,251 ± 7126
Triglycerides, mg/dL × 260 min	22,263 ± 4641	44,624 ± 9404	44,909 ± 5670
Insulin, μU/mL × 260 min	8783 ± 1795[b]	22,781 ± 2307	15,812 ± 3198

[1]All values are means ± SEMs. [a,b]ANOVA with Bonferroni adjustment for multiple comparisons: [a]vs. T2Ds and [b]vs. OBEs, $P < 0.05$. CON, young, lean, insulin-sensitive human; FFA, free fatty acid; OBE, insulin-resistant, glucose-tolerant, obese human; T2D, type 2 diabetes patient.

related to complex I was 53% lower ($P = 0.01$) in T2Ds ([but not in OBEs ($P = 0.15$)] than in CONs (Figure 2C). State 3 respiration related to complexes I and II and the maximal uncoupled respiratory capacity (state u) were not different between groups. In comparison with CONs, intramyocellular lipids were 3-fold higher ($P = 0.01$) only in T2Ds (Figure 2D).

Correlations

The positive relation of fasting hepatic γATP with fasting and postprandial hepatic Pi at 240 min remained after adjustment for the group effect (fasting Pi: $r = 0.62$, $P < 0.001$; postprandial Pi: $r = 0.41$, $P = 0.03$). The positive correlation between fasting hepatic γATP and muscle state 3 respiration coupled to complexes I and II (**Table 4**) remained significant after adjustment for the group effect ($r = 0.40$, $P = 0.05$). The inverse relations of fasting Pi with BMI, waist circumference, triglycerides, FFAs, and HCLs as well as the positive relation with M value disappeared after adjustment for the group effect (Table 4). Neither fasting γATP nor Pi was correlated ($P > 0.15$) with serum uric acid (Table 4).

Postprandial Pi at 160 min was negatively associated with HCLs ($r = −0.55$, $P = 0.002$) and triglycerides ($r = 0.38$, $P = 0.048$) even after adjustments. Postprandial Pi at 240 min was correlated positively with muscle state 3 respiration coupled to complexes I and II ($r = 0.41$, $P = 0.049$) after adjustments. The positive correlation between state 3 respiration coupled to complex I and M value ($r = 0.46$, $P = 0.02$) disappeared after adjustment for group effects. Neither state 3 respiration coupled to complexes I and II nor state u respiration was related ($P > 0.8$) to M value.

DISCUSSION

This study showed that the ingestion of one single meal already increased hepatic γATP and Pi concentrations in young, obese, insulin-resistant humans but not in elderly obese T2Ds or in young, lean, healthy humans. These postprandial changes of hepatic energy metabolism did not lead to rapid alterations in the hepatic lipid content and were not related to insulin sensitivity. Furthermore, the fasting muscle oxidative capacity was lower only in T2Ds.

Almost all previous studies that assessed hepatic energy metabolism in vivo were performed in postabsorptive humans (18, 29–31). With the use of an intravenous fructose challenge (29, 32, 33), the few previous dynamic ^{31}P-MRS studies reported ATP recovery to be unchanged in the obese (33) and

reduced in advanced NAFLD patients (29). Although these studies assessed the submaximal OXPHOS capacity, the current study showed supply-driven changes in γATP after meal intake.

In the OBEs group, transient postprandial increases in insulin and FFA flux into the liver because of the impaired suppression of lipolysis may have collectively promoted postprandial hepatic γATP synthesis. The greater lipid availability likely stimulated lipid oxidation with upregulated γATP production. In the postabsorptive state, the upregulation of hepatic oxidative capacity (13) and the expression of hepatic OXPHOS genes (13, 34) have been previously shown in liver biopsies of obese humans. In contrast, higher prevailing insulin would favor lipogenesis, which is an energy-demanding process that drives hepatic energy metabolism. In line with this effect, OBEs showed at least a trend toward a greater postprandial rise in Pi, which is known to stimulate ATP synthase flux in the human liver (30). Postprandial Pi concentrations were further related inversely to HCLs, which suggested that greater postprandial hepatic mitochondrial metabolism may prevent the progression of steatosis. Nevertheless, hyperinsulinemia could have also caused the postprandial Pi rise by promoting Pi uptake as shown in cultured hepatocytes (35).

TABLE 3
Fasting hepatic γATP and Pi concentrations before and after mixed-meal ingestion by CONs ($n = 10$), OBEs ($n = 10$), and T2Ds ($n = 10$)[1]

	CONs	OBEs	T2Ds
γATP, mmol/L			
Fasting	2.3 ± 0.2	1.9 ± 0.1[a,b]	2.0 ± 0.2[b]
160 min	2.3 ± 0.1	2.3 ± 0.2	2.3 ± 0.2
240 min	2.3 ± 0.1	2.4 ± 0.1	2.4 ± 0.2
Pi, mmol/L			
Fasting	2.2 ± 0.2[c,d]	1.3 ± 0.1[b]	1.5 ± 0.1
160 min	2.1 ± 0.1[c,d]	1.4 ± 0.2	1.6 ± 0.2
240 min	2.3 ± 0.1[c,d]	1.7 ± 0.1	1.7 ± 0.2

[1]All values are means ± SEMs. A generalized 2-factor ANOVA without Bonferroni adjustment for multiple comparisons was used. P values for time-group interaction values were 0.44 and 0.75 for the γATP model and the Pi model, respectively. Fasting hepatic γATP did not differ between groups. [a,b]Fasting γATP or Pi vs. γATP or Pi: [a]at 160 min and [b]at 240 min, $P < 0.05$. [c,d]Significances of differences for fasting γATP or Pi vs. γATP or Pi at 160 or 240 min were lost after Bonferroni adjustment for multiple comparisons: [c]vs. T2D and [d]vs. OBE, $P < 0.05$. After Bonferroni adjustment, fasting hepatic Pi was lower only in OBEs ($P = 0.02$) compared with CONs. CON, young, lean, insulin-sensitive human; OBE, insulin-resistant, glucose-tolerant, obese human; Pi, inorganic phosphate; T2D, type 2 diabetes patient.
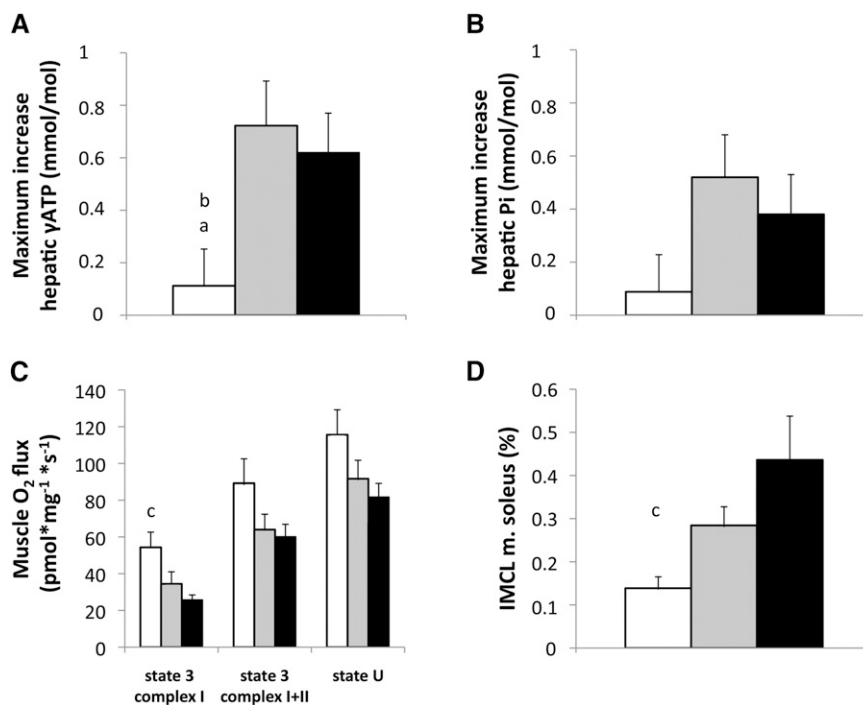
FIGURE 2 Mean ± SEM maximum postprandial increases of hepatic γATP (A) and Pi (B) as well as of fasting muscle oxidative capacity (C) and the IMCL (D) in the soleus muscle of CONs (white columns), OBEs (gray columns), and T2Ds (black columns). Hepatic γATP and Pi: $n = 10$ per group. Muscle oxidative capacity: CONs, $n = 10$; OBEs, $n = 7$; and T2Ds, $n = 9$. IMCL: CONs, $n = 8$; OBEs, $n = 8$; and T2Ds, $n = 9$. [a,b,c]One-factor ANOVA with Bonferroni adjustment: [a]$P = 0.03$ CONs vs. OBEs, [b]$P = 0.09$ CONs vs. T2Ds, [c]$P = 0.01$ CONs vs. T2Ds. Data for the whole-body insulin sensitivity and muscle oxidative capacity of 9 CONs, 6 OBEs, and 7 T2Ds have been published previously (7, 16). CON, young, lean, insulin-sensitive human; IMCL, intramyocellular lipid content; OBE, insulin-resistant, glucose-tolerant, obese human; T2D, type 2 diabetes patient.

The elderly, obese T2Ds did not exhibit significant changes in postprandial hepatic γATP despite a similar degree of insulin resistance and the suppression of lipolysis as in the young OBEs group. Prolonged hyperglycemia and hyperinsulinemia as well as an increased hepatic lipid availability under conditions of chronic insulin resistance could have progressively impaired the hepatic mitochondrial flexibility because of mechanisms related to glucolipotoxicity and oxidative stress (9, 36). As shown recently, an upregulated fasting hepatic oxidative capacity exists in the presence of impaired mitochondrial efficiency and antioxidant defense, which may ultimately reduce the hepatic oxidative capacity in patients with higher degrees of glycemia, lipidemia, and inflammatory NAFLDs (13). Although the current findings are in agreement with previous human (13) and rodent (37, 38) studies, the

studies assessed different mitochondrial features under different nutritive conditions.

In the current study, fasting hepatic Pi was 30–40% lower in both insulin-resistant groups, which was similar to the reduction by 20–30% in other insulin-resistant cohorts (18, 30) or by 30–50% in alcohol- or hepatitis C–related liver disease (39, 40). Fasting hepatic γATP did not differ between young obese and lean humans in contrast to one previous study that showed a reduced hepatic ATP content in overweight and obese humans (33). The latter study provided no information on insulin sensitivity and hepatic steatosis, which can determine fasting hepatic ATP. Fasting hepatic γATP only tended to be lower in the elderly, obese T2Ds than in the young, lean humans. Of note, a marked reduction in hepatic γATP concentrations (18) or ATP synthase flux (30) was reported only in age- and BMI-matched

TABLE 4
Correlation analyses for fasting hepatic phosphorus metabolites[1]

	BMI	Waist	Hip	Triglycerides	FFAs	Uric acid	M value	HCLs	State 3, C I+II
n	30	30	30	30	30	30	30	30	26
γATP									
r	−0.30	−0.18	−0.38	−0.12	−0.24	0.05	0,15	−0.02	0.48
P	0.11	0.33	0.038	0.55	0.18	0.79	0.42	0.88	0.02
Pi									
r	−0.56	−0.44	−0.51	−0.40	−0.50	−0.10	0.36	−0.40	0.34
P	0.001	0.002	0.004	0.027	0.005	0.62	0.008	0.030	0.089

[1]Pearson correlation coefficients are given for the correlation between hepatic γATP or Pi with BMI, waist, hip, triglycerides, FFAs, M value, HCLs, and muscle oxidative capacity (State 3 CI+II). Data for M value and muscle oxidative capacity of 6 OBEs, 7 T2Ds, and 9 CONs were published previously (7, 16). FFA, free fatty acid; HCL, hepatocellular lipid; M value, whole-body insulin sensitivity; Pi, inorganic phosphate; State 3 C I+II, state 3 complexes I and II.

T2Ds and control subjects. Although some possible determinants of hepatic energy metabolism such as age, long-term glycemic and lipid control, and HCLs were comparable in T2Ds of this and previous studies, fasting glucose was higher in T2Ds of a previous study (30). Therefore, glucose toxicity may have accounted for the lower hepatic ATP synthase flux in those patients. In addition, the rather short known diabetes duration (5.7 y) in the current study suggested a shorter exposure to hyperglycemia and, thereby, could explain the absence of lower fasting γATP. Finally, serum uric acid concentrations, which possibly reflected inflammation or dietary fructose consumption (32), were, to our knowledge, not assessed in previous studies but were not associated with hepatic energy metabolism in the current study.

This study also showed that fasting hepatic γATP was correlated with the fasting muscle oxidative capacity (i.e., state 3 respiration coupled to complexes I and II) in the total cohort. This finding suggests similar relations of hepatic energy metabolism with HCLs and insulin sensitivity as reported for muscle ATP synthase flux (41). However, only fasting hepatic Pi exhibited a correlation with insulin sensitivity and HCLs, which was lost after adjustment for group effects. Measurements of energy metabolism differed by the method used (noninvasive vs. invasive) and by the tested mitochondrial feature (end product of ATP synthase flux vs. maximum electron transport chain activity). Specifically, the measurement in the liver was done during physiologic changes in the substrate supply and demand, whereas the muscle was examined in the presence of an excessive substrate supply. Nevertheless, this comparison provides a preliminary support of the concept of tissue-specific differences in mitochondrial function independent of insulin sensitivity. Obese T2Ds had lower muscle respiration than that of lean volunteers but tended to have greater hepatic γATP increases, in line with the transient increase of hepatic but early decrease of muscle mitochondrial function in mouse models (38). Nevertheless, this conclusion requires caution because of the differences in methods and metabolic conditions applied to assess hepatic and muscle energy metabolism.

The strengths of this study resided in the monitoring of hepatic energy metabolism in vivo with the use of state-of-the-art ^{31}P-MRS and the comparison with muscle energy metabolism ex vivo. Nevertheless, the group differences in anthropometric and metabolic features limited the generalizability of the results. Specifically, the difference between OBEs and T2Ds vs. CONs in the maximum hepatic γATP increase may have had different causes in these groups. First, the γATP increase in T2Ds vs. CONs could have been due to a higher substrate (glucose and FFAs) delivery, portal blood flow, older age, or metformin treatment. Changes in substrate delivery may only reflect the metabolic diversity of the groups, but they affect the substrate oxidation fueling tricarboxylic acid cycle and OXPHOS for ATP synthesis (42). Hyperglycemia and hyperinsulinemia can increase portal blood flow and substrate extraction, which, however, is not necessarily altered in T2Ds (19). As to possible age-dependent effects reported for muscle mitochondria in elderly humans (43), correlation analyses did not identify age as a predictor of changes in hepatic ATP turnover (18, 30). Metformin was withdrawn for 3 d as in previous studies (4, 5, 18, 19) and does not acutely affect he cellular energy charge but, rather,

inhibits the hepatic glycerophosphate shuttle (44). Moreover, complex 1 respiration is not inhibited in the muscle of metformin-treated T2Ds (45). Second, the γATP increase in OBEs vs. CONs could have been due to effects related to inflammation that were suggested from increased ALT and uric acid concentrations. The observation that obese patients with inflammatory NAFLDs have a lower fasting hepatic oxidative capacity does not support this contention (13). Finally, despite a higher substrate delivery in T2Ds, hepatic γATP and Pi concentrations increased only in OBEs. Thus, if altered substrate flux critically affects postprandial energy metabolism, the observed alterations in OBEs would have been underestimated rather than overestimated.

In conclusion, young obese humans but not elderly T2Ds exhibit higher increase of prandial hepatic γATP concentrations independently of insulin resistance. These results provide in vivo evidence for an obesity-associated postprandial adaptation of hepatic energy metabolism. Moreover, elderly T2Ds, but not glucose tolerant, obese, and equally insulin-resistant humans, exhibit impaired fasting muscle energy metabolism. These findings support the concept of a differential tissue-specific regulation of energy metabolism, which may occur independent of insulin resistance.

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