Free Fatty Acids Increase Hepatic Glycogen Content in Obese Males

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Obesity is associated with increased hepatic glycogen content. In vivo and in vitro data suggest that plasma free fatty acids (FFA) may cause this increase. In this study we investigated the effect of physiological plasma FFA levels on hepatic glycogen metabolism by studying intrahepatic glucose pathways in lean and obese subjects. Six lean and 6 obese males were studied twice during a 16- to 22-hour fast, once with and once without acipimox, an inhibitor of lipolysis. Intrahepatic glucose fluxes were measured by infusion of [2-$^{13}C_1$]glycerol, [1-$^{2}H_1$]galactose, and [U-$^{13}C_6$]glucose. Acetaminophen was administered as a glucuronate probe. In both lean and obese control studies, plasma FFA levels increased progressively, whereas acipimox completely suppressed plasma FFA levels for the whole study period. In lean males glycogenolysis did not change in the acipimox study, but decreased in the control study ($P < .01$). In lean males, neither glycogen synthesis, glycogen synthesis retained as glycogen, nor glycogen balance differed between control and acipimox studies. In obese males glycogenolysis did not change in the acipimox study, but decreased in the control study ($P < .01$). Glycogen synthesis did not change in either study. Glycogen synthesis retained as glycogen did not change in acipimox study, but increased in the control study ($P = .03$). Glycogen balance did not change in the acipimox study, but increased in the control study ($P < .01$). This study demonstrates that in obese males physiological levels of FFA contribute to the retention of hepatic glycogen during short-term fasting by inhibiting breakdown of glycogen and increasing glycogen synthesis retained as glycogen, whereas in lean males this effect was absent due to unaltered glycogen synthesis retained as glycogen.

OBESITY IN HUMANS is characterized by increased hepatic glycogen content.[1] These data obtained in obese humans are consistent with those obtained in dietary obese rats which demonstrate increased glycogen content after a 24-hour fast.[2] The pathophysiology behind this increased content is unknown.

In the postabsorptive state, hepatic glycogen is an important source for plasma glucose. The availability of hepatic glycogen is controlled by several factors, including free fatty acids (FFA). During fasting, lipolysis is enhanced, plasma FFA levels rise and glucose production adapts by maintenance of gluconeogenesis and a decrease in the rate of glycogenolysis.[3] Stingl et al, using ^{13}C nuclear magnetic resonance (NMR) spectroscopy, found that lipid/heparin infusion restrained the breakdown of hepatic glycogen content in fasting lean males.[4] Accordingly, in vitro studies have shown that FFA increase hepatic glycogen content by activation of glycogen synthase and inhibition of glycogen phosphorylase. The latter is inhibited by increased glucose-6-phosphate from gluconeogenesis and by adenosine triphosphate (ATP) derived from FFA oxidation.[5] These in vitro data in rodents suggest that FFA influence glycogen content by simultaneously stimulating synthesis and inhibiting breakdown of glycogen.

Although the effects of FFA on hepatic glycogen content seem evident, no in vivo data on the influence of physiological FFA levels on intrahepatic glycogen metabolism fluxes in humans, lean or obese, are available. Therefore, this study examines the effects of physiological FFA levels on hepatic glycogen metabolism by measuring intrahepatic glucose pathways using stable isotope techniques in lean and obese males during short-term fasting.

MATERIALS AND METHODS

Subjects

Six healthy lean male volunteers (age, 32 ± 4 years; weight, 68 ± 2 kg; height, 1.70 ± 0.02 m; body mass index, 22.6 ± 0.74 kg/m^2) and 6 obese male volunteers (age, 58 ± 2 years; weight, 96 ± 5 kg; height, 1.81 ± 0.02 m; body mass index, 29.1 ± 0.9 kg/m^2) were included in a cross-over, saline-controlled study after obtaining written informed consent. None of the subjects had experienced any febrile disease in the month prior to the study or used medication. For 3 days prior to the study, all volunteers consumed a weight-maintaining diet containing at least 250 g of carbohydrates. The study was approved by the Medical Ethical Committee of the Academic Medical Center in Amsterdam.

Study Protocol

The study protocol is depicted in Fig 1. Each subject was studied twice, once with and once without acipimox. Both study protocols were performed in balanced assignment and separated by at least 4 weeks. The subjects were fasted from 6 PM on the day prior to the study until the end of the study. During the studies subjects were confined to bed. At 6:45 AM (t = −3:00 h), a catheter was placed into an antecubital vein for infusion of stable isotope tracers. Another catheter was inserted retrogradely into a contralateral hand vein kept in a thermoregulated (60°C) plexiglas box for sampling of arterialized venous blood. In all studies saline was infused as NaCl 0.65% at a rate of 50 mL/h to keep the catheters patent. [2-$^{13}C_1$]glycerol, [1-$^{2}H_1$]galactose, and [U-$^{13}C_6$]glucose were infused as tracers. Before starting tracer infusion,

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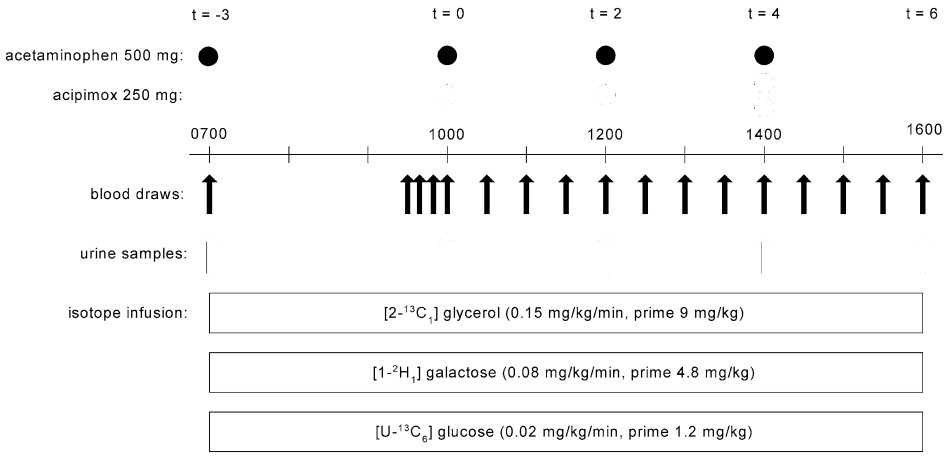

Fig 1. Isotope infusion and sampling protocol.

blood was sampled for determination of background enrichment at t = −3:00 h. Then, a primed continuous infusion of each isotope was started: [2-$^{13}C_1$]glycerol (prime, 9 mg/kg; continuous, 0.15 mg/kg/min), [1-$^{2}H_1$]galactose (prime, 4.8 mg/kg; continuous, 0.08 mg/kg/min), and [U-$^{13}C_6$]glucose (prime, 1.2 mg/kg; continuous, 0.02 mg/kg/min) and continued until the end of the study at t = 6:00 h. Acetaminophen (500 mg; Centrafarm Services, Etten-Leur, The Netherlands) was given orally at t = −3:00, 0:00, 2:00, and 4:00 h. In the intervention study, acipimox was administered orally t = 0:00 (250 mg), t = 2:00 (250 mg), and t = 4:00 (500 mg).

At t = −20 minutes, −15 minutes, −10 minutes, and t = 0:00 h, blood samples were drawn for determination of steady state isotopic enrichment. At t = 0:00 h, blood samples for baseline values of glucoregulatory hormones and FFA were drawn.

Every 30 minutes between t = 0:00 h and the end of the study at t = 6:00 h, blood samples were drawn for measurement of isotopic enrichment of glucose by [U-$^{13}C_6$]glucose to determine glucose turnover. Isotopic enrichment of glucose and acetaminophen-glucuronate (GlcUA) by [2-$^{13}C_1$]glycerol, [1-$^{2}H_1$]galactose was measured every 2 hours between t = 0:00 h and t = 6:00 h. Blood samples for measurement of glucoregulatory hormones were drawn every 30 minutes. Blood samples for measurement of FFA were drawn every hour.

Fat free mass (FFM) was calculated using Hume's gender-specific formula.

Chemicals, Isotopes, and Gas Chromatography-Mass Spectrometric Analyses

[2-$^{13}C_1$]glycerol, [1-$^{2}H_1$]galactose, and [U-$^{13}C_6$]glucose were purchased from Cambridge Isotopes (ARC Laboratories, Amsterdam, The Netherlands). Isotopes were greater than 98% pure and greater than 99% enriched. Isolation of metabolites, preparation for mass spectrometry, and mass spectrometric analyses were done as previously described.[6] The gas chromatography column used was a 60 m × 0.25 mm × 0.25 μm DB17 capillary column (J&W Scientific, Folsom, CA) on a Hewlett-Packard 5890 Series II gas chromatograph coupled to an HP 5989 A model mass spectrometer (Hewlett Packard, Palo Alto, CA).

Pathways of Glucose Metabolism

The pathways of glucose metabolism are shown in Fig 2. During short-term starvation (<24 hours), plasma glucose originates from gluconeogenesis and glycogen breakdown. Gluconeogenic flux can be directed towards plasma directly (direct gluconeogenesis), or towards glycogen by passing through the uridine diphosphate (UDP)-glucose pool (indirect gluconeogenesis). The sum of direct and indirect gluconeogenesis, corrected for duplicated fluxes, equals total flux through the gluconeogenic pathway[6] (not further discussed in this report) and includes gluconeogenic glucose, which is retained in hepatic glycogen. Absolute gluconeogenesis is the gluconeogenesis flux that appears in plasma glucose. Absolute glycogenolysis is the difference between endogenous glucose production and absolute gluconeogenesis. Plasma glucose from glycogen originates from pre-existing hepatic glycogen and indirect gluconeogenesis through the glycogen pool. Liver glycogen is synthesized from plasma glucose and from indirect gluconeogenic flux. Newly synthesized glycogen, ie, glycogen synthesized during the course of the study, can be either retained in the liver (glycogen synthesis retained as glycogen) or be degraded again rapidly. All glycogen synthesis flux per se originates from the UDP-glucose pool, due to the in vivo irreversibility of the glucose-1-phosphate to UDP-glucose reaction. Glycogen synthesis flux can therefore be calculated as the sum of glucose appearance from UDP-glucose (glycogen synthesis flux and subsequent breakdown) and glycogen synthesis retained as glycogen (originating from both plasma glucose and indirect gluconeogenesis). Glycogen balance is calculated by subtracting absolute glycogenolysis from glycogen synthesis retained as glycogen. Glycogen balance is considered a better measure for net glycogen breakdown than absolute glycogenolysis, for the latter overestimates the glycogen contribution to plasma glucose since it does not correct for glycogen synthesis retained as glycogen[6] (Fig 2).

Calculations of Metabolic Fluxes

Calculations were done with mass isotopomer distribution analysis (MIDA) and the secreted glucuronate technique as previously described by Hellerstein et al.[6-8]

Briefly, the pathways by which glucose and gluconeogenic fluxes were converted to glycogen were traced by use of [2-$^{13}C_1$]glycerol, [1-$^{2}H_1$]galactose, and [U-$^{13}C_6$]glucose, and acetaminophen as a noninvasive xenobiotic probe of intrahepatic UDP-glucose. Secreted glucuronate-acetaminophen (GlcUA) was sampled in urine and used to determine UDP-glucose enrichment. Six primary measurements were made in plasma glucose and GlcUA. Endogenous glucose production (EGP) was calculated from the dilution of infused [U-$^{13}C_6$]glucose in plasma M_6-glucose. The rate of appearance of hepatic UDP-glc was calculated from the dilution of [1-$^{2}H_1$]galactose in M_1-GlcUA isolated from urinary acetaminophen-GlcUA. Fractional gluconeogenesis to plasma glucose was calculated by MIDA from [2-$^{13}C_1$]glycerol in plasma glucose. The fractional gluconeogenesis to hepatic UDP-glc was calculated by MIDA from [2-$^{13}C_1$]glycerol in GlcUA isolated from urinary acetaminophen-GlcUA. The fractional direct pathway contribution from plasma glucose to hepatic UDP-glc was calculated by the

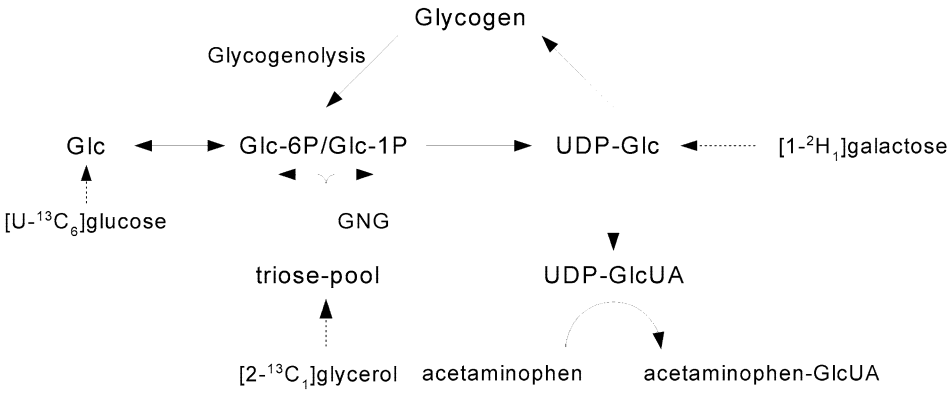

Fig 2. Intrahepatic pathways of glucose metabolism, glucuronate and stable isotopes.

precursor-product relationship from M_6-glucose to M_6-GlcUA. The fractional recovery of labeled hepatic UDP-glc in plasma glucose was calculated from the proportion of infused [1-$^2H_1$]galactose that appeared in plasma glucose. By combining these primary measurements, several secondary parameters could be derived. These included rates of absolute, direct, and indirect gluconeogenesis, and rates of absolute glycogenolysis, glycogen synthesis, glycogen synthesis retained as glycogen, and glycogen balance.

Assays

All measurements in each individual subject were performed in the same run, and all samples were tested in duplicate. Glucose concentrations were measured on a Beckman glucose analyzer (Beckman, Palo Alto, CA). Plasma insulin concentration was determined by radioimmunoassay (RIA) (Insulin RIA 100; Pharmacia Diagnostic, Uppsala, Sweden): intra-assay coefficient of variation (CV) 3% to 5%, interassay CV 6% to 9%, detection limit 12 pmol/L (2 mU/L). C-peptide was measured by RIA (RIA-coat C-peptide; Byk-Sangtec Diagnostica, Dietzenbach, Germany): intra-assay CV 4% to 6%, interassay CV 6% to 8%, detection limit 0.05 nmol/L. Glucagon was determined by RIA (Linco Research, St Charles, MO): intra-assay CV 3% to 5%, interassay CV 9% to 13%, detection limit 15 ng/L. Cortisol was measured by enzyme-immunoassay on an Immulite analyzer (DPC, Los Angeles, CA): intra-assay CV 2% to 4%, interassay CV 3% to 7%, detection limit 50 nmol/L. Catecholamines were measured by an in-house high-performance liquid chromatography HPLC method—norepinephrine: interassay CV 6% to 8%, intra-assay CV 7% to 10%, detection limit 0.05 nmol/L; and epinephrine: interassay CV 6% to 8%, intra-assay CV 7% to 12%, detection limit 0.05 nmol/L. Plasma FFA were measured by an enzymatic method (NEFAC, Wako Chemicals, Neuss, Germany): intra-assay CV 2% to 4%, interassay CV 3% to 6%, detection limit 0.02 mmol/L.

Calculations and Statistics

Because plasma glucose concentrations and tracer/tracee ratios remained constant during each sampling phase of the study, calculations for steady-state kinetics were applied, adapted for the use of stable isotopes.[9,10] To correct for differences in fat mass, fluxes are expressed for kilogram FFM. The overall differences in the parameters between the acipimox and control studies were tested using analysis of repeated measures by means of the proc mixed procedure of the SAS software package (version 6.12; SAS Institute, Cary, NC). For each parameter and for each group the regression coefficient was obtained from the same model in which time was entered as a continuous variable. The acipimox effect was calculated by subtracting data obtained from the acipimox studies from data obtained from the control studies with adjustment for differences between results at t = 0. The differences of acipimox effect between the lean and obese groups were analyzed using the Mann-Whitney test (SPSS 11.5; SPSS Inc, Chicago, IL). Results are presented as means ± SEM.

RESULTS

Lean Subjects

Control study (Table 1). After an initial decrease, plasma FFA increased between t = 1:00 h and the end of the study ($P < .01$). Between t = 0:00 h and t = 6:00 h plasma glucose concentration and glucose production decreased (both $P < .01$). Absolute gluconeogenesis did not change (Fig 3A). Neither direct gluconeogenesis nor indirect gluconeogenesis (Fig 3C and E) changed. Absolute glycogenolysis decreased ($P < .01$) (Fig 4A). Neither glycogen synthesis nor glycogen synthesis retained as glycogen changed (Fig 4C). Due to the decrease in glycogen breakdown, glycogen balance became less negative over time ($P < .01$) (Fig 4E).

Acipimox study (Table 1). Plasma FFA decreased between t = 0:00 h and t = 6:00 h ($P < .01$), decreasing 75% within the first hour after acipimox administration ($P < .01$, *t* test). Plasma glucose concentration decreased ($P < .01$). Glucose production did not decrease with acipimox. Absolute gluconeogenesis showed a transient decrease from t = 0:00 to t = 4:30 h, but returned to baseline at t = 6:00 h (Fig 3A). Direct gluconeogenesis remained unchanged after a transient decrease between t = 0:00 to t = 4:00 h (Fig 3C). Indirect gluconeogenesis did not change (Fig 3E). Absolute glycogenolysis, glycogen synthesis, and glycogen synthesis retained as glycogen did not change (Fig 4A and C). Glycogen balance was unchanged due to persistent glycogen breakdown (Fig 4E).

Acipimox versus control study (Table 1). Plasma FFA were significantly lower in the acipimox study than in the control study from t = 1:00 onwards ($P < .01$). Plasma glucose concentrations were not different between studies. Although glucose production decreased in the control study and did not change in the acipimox study, there was no significant difference between curves in the course over time. Absolute, direct, and indirect gluconeogenesis with acipimox were not different from control (Fig 3A, C, and E). Breakdown of glycogen was higher with acipimox than in the control study ($P = .04$) (Fig 4A). Glycogen synthesis and glycogen synthesis retained as glycogen were not different between the control and acipimox

Table 1. FFA Concentrations (mmol/L), Glucose Concentrations (mmol/L), and Intrahepatic Fluxes of Glucose Metabolism (μmol/kg/min) in Lean and Obese Males After 16 (t = 0) and 22 (t = 6) Hours of Fasting in Control and Acipimox Studies

		Lean			Obese			Lean *v* Obese
Variable	Study	t = 0	t = 6	*P**	t = 0	t = 6	*P**	*P*†
Free fatty acids	Control	0.53 ± 0.09	0.54 ± 0.07	<.01	0.51 ± 0.03	0.65 ± 0.04	<.01	NS
	Acipimox	0.52 ± 0.05	0.06 ± 0.01		0.48 ± 0.05	0.10 ± 0.02		
Glucose concentration	Control	5.28 ± 0.14	4.57 ± 0.19	NS	6.09 ± 0.19	5.40 ± 0.13	NS	NS
	Acipimox	5.62 ± 0.13	4.87 ± 0.25		6.30 ± 0.17	5.46 ± 0.11		
Glucose production	Control	21.28 ± 1.22	17.17 ± 1.22	NS	19.89 ± 0.50	15.22 ± 0.39	<.01	NS
	Acipimox	20.39 ± 1.06	18.94 ± 0.83		18.72 ± 0.67	17.28 ± 0.50		
Absolute gluconeogenesis	Control	5.28 ± 0.44	6.17 ± 0.39	NS	4.89 ± 0.39	6.33 ± 0.22	<.01	NS
	Acipimox	5.00 ± 0.61	4.89 ± 0.50		4.50 ± 0.22	4.28 ± 0.28		
Direct gluconeogenesis	Control	4.72 ± 0.50	5.33 ± 0.39	NS	4.56 ± 0.39	5.56 ± 0.22	<.01	NS
	Acipimox	4.39 ± 0.67	4.67 ± 0.61		4.17 ± 0.22	3.72 ± 0.28		
Indirect gluconeogenesis	Control	1.28 ± 0.28	1.56 ± 0.17	NS	0.72 ± 0.06	1.72 ± 0.11	<.01	NS
	Acipimox	1.17 ± 0.22	1.00 ± 0.17		0.78 ± 0.06	1.06 ± 0.17		
Absolute glycogenolysis	Control	16.00 ± 0.94	11.00 ± 1.28	.04	15.00 ± 0.61	8.89 ± 0.56	<.01	NS
	Acipimox	15.44 ± 0.67	14.06 ± 0.94		14.22 ± 0.67	13.00 ± 0.44		
Glycogen synthesis	Control	4.22 ± 0.39	3.78 ± 0.39	NS	3.44 ± 0.28	3.94 ± 0.22	NS	NS
	Acipimox	4.06 ± 0.56	3.39 ± 0.61		3.61 ± 0.28	3.61 ± 0.17		
Glycogen synthesis rag‡	Control	1.39 ± 0.33	1.61 ± 0.28	NS	0.67 ± 0.11	1.44 ± 0.11	.03	.01
	Acipimox	1.06 ± 0.17	1.28 ± 0.17		0.78 ± 0.06	1.06 ± 0.11		
Glycogen balance	Control	−14.61 ± 0.83	−9.67 ± 1.39	NS	−14.33 ± 0.61	−7.44 ± 0.61	<.01	.01
	Acipimox	−14.39 ± 0.56	−12.50 ± 1.00		−13.44 ± 0.67	−11.94 ± 0.50		

*P value between curves of acipimox and control studies over time.

†P value of acipimox effect between lean and obese studies.

‡Glycogen synthesis retained as glycogen.

Abbreviation: NS, not significant.

study (Fig 4C). Glycogen balance did not show any difference between the control and acipimox study (Fig 4E).

Counterregulatory hormones (Table 2). Both insulin and C-peptide decreased in the acipimox and control study, but more so in the acipimox than in the control study. Glucagon increased in the last 30 minutes of the acipimox study, but did not change in the control study. Cortisol levels were not different. Epinephrine increased during the last 2 hours of the acipimox study, but did not change in the control study. Norepinephrine was not different between the acipimox and control study.

Obese Subjects

Control study (Table 1). Plasma FFA increased between t = 0:00 and t = 6:00 ($P < 0.01$). Plasma glucose concentration and glucose production decreased (both $P < .01$). Absolute, direct, and gluconeogenesis increased (all $P < .01$) (Fig 3B, D, and F). Absolute glycogenolysis decreased ($P < .01$) (Fig 4B). Glycogen synthesis did not change. Glycogen synthesis retained as glycogen increased ($P < .01$) (Fig 4D). Due to the decrease in glycogen breakdown and increase in glycogen synthesis retained as glycogen, glycogen balance became less negative over time ($P < .01$) (Fig 4F).

Acipimox study (Table 1). Plasma FFA decreased 79% within 1 hour ($P < .01$) and remained suppressed until t = 6:00. Between t = 0:00 and t = 6:00 plasma glucose concentration and glucose production decreased (respectively, $P < .01$ and $P = .04$). Absolute gluconeogenesis did not change (Fig 3B). While direct gluconeogenesis did not change, indirect gluconeogenesis increased ($P = .01$) (Fig 3D and F). Neither absolute glycogenolysis nor glycogen synthesis changed (Fig 4B). Glycogen synthesis retained as glycogen increased ($P = .03$) (Fig 4D). Glycogen balance remained stable (Fig 4F).

Control versus acipimox study (Table 1). Plasma FFA were lower in the acipimox study than in the control study ($P < .01$). Plasma glucose concentrations were not different. Glucose production was higher in the acipimox study than in the control study ($P < .01$). Absolute, direct, and indirect gluconeogenesis with acipimox were lower than in the control study (all $P < .01$) (Fig 3B, D, and F). Absolute glycogenolysis was higher in the acipimox study than in the control study ($P < .01$) (Fig 4B). Glycogen synthesis was not different between studies. Glycogen synthesis retained as glycogen was lower in the acipimox study than in the control study ($P = .02$) (Fig 4D). Glycogen balance was more negative over time in the acipimox study than in the control study ($P < .01$) (Fig 4F).

Glucoregulatory hormones (Table 2). Both insulin and C-peptide decreased in the acipimox and control study, but were lower in the acipimox than in the control study. Glucagon was not different between the acipimox and control studies. Cortisol levels were not different. Epinephrine increased during the last hour in the acipimox study, resulting in a difference between acipimox and control studies ($P < .01$). Norepinephrine was not different between the acipimox and control study.

Lean Versus Obese Subjects

The acipimox effect between lean and obese subjects was different for glycogen synthesis retained as glycogen and gly-

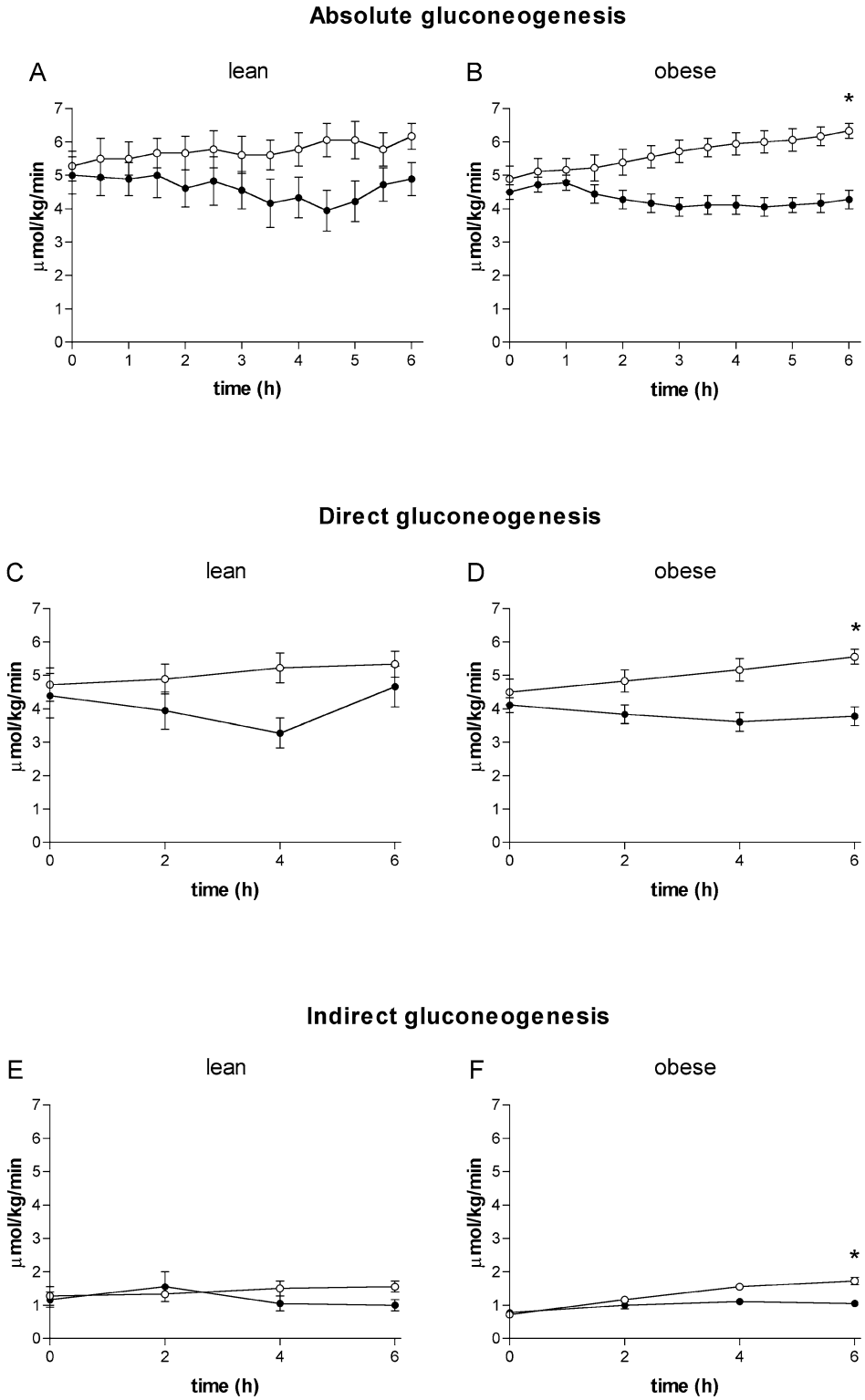

Fig 3. Absolute, direct, and indirect gluconeogenesis in lean and obese males between 16 (t =0) and 22 (t =6) hours of fasting. Control study (○) and acipimox study (●).*$P < .05$ between curves of control and acipimox studies over time.

cogen balance (both $P = .01$); these effects of acipimox were higher in obese than in lean subjects (Table 1). No difference of acipimox effect was found between lean and obese subjects for other pathways contributing to glycogen content.

DISCUSSION

This study shows that during short-term fasting FFA influences glycogen content in a different way in obese and lean

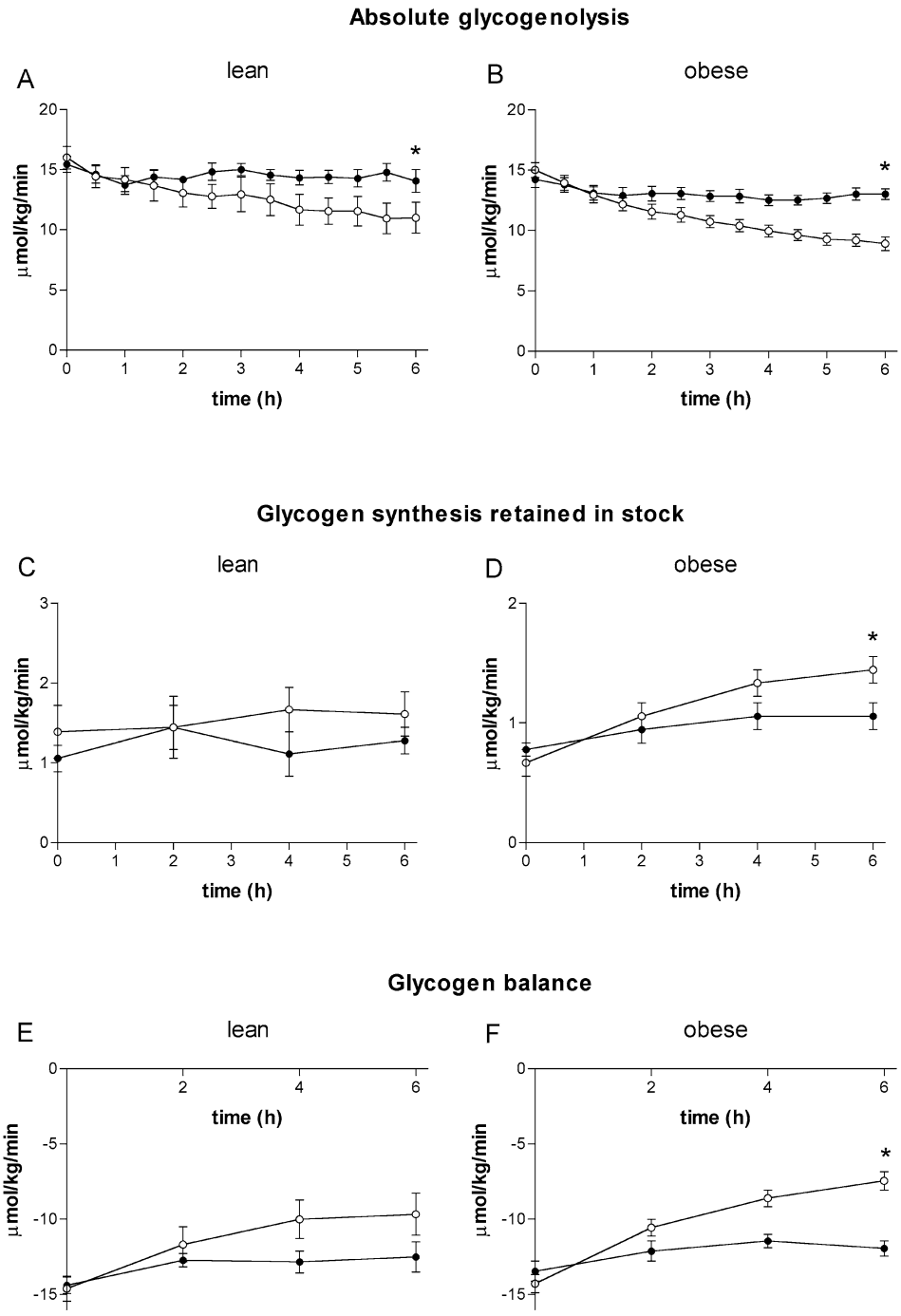

Fig 4. Glycogenolysis, glycogen synthesis retained as glycogen and glycogen balance in lean and obese males between 16 (t =0) and 22 (t =6) hours of fasting. Control study (○) and acipimox study (●).*$P < .05$ between curves of control and acipimox studies over time.

subjects. In obese males, physiological plasma FFA levels contribute to the retention of hepatic glycogen content by inhibiting breakdown of glycogen and by increasing glycogen synthesis retained as glycogen. However, in lean males physiological plasma FFA levels did not affect retention of glycogen content; FFA did inhibit glycogen breakdown, but did not affect glycogen synthesis retained as glycogen.

There is no golden standard available for the measurement of gluconeogenesis and fluxes through UDP-glucose and glycogen in vivo in humans. All available (isotopic) techniques come with assumptions and technical imperfections. The technique we used to measure intrahepatic carbohydrate metabolic fluxes was developed by Hellerstein et al and combines MIDA, the secreted glucuronate technique and the standard isotope dilution technique.[6-8,10,11] The glucuronate technique and MIDA rely on the validity of a number of biochemical key conditions. The technical backgrounds and the basis for calculations including the assump-

Table 2. Glucoregulatory Hormones in Lean and Obese Males After 16 (t = 0) and 22 (t = 6) Hours of Fasting in Control and Acipimox Studies

		lean			obese		
Variable	Study	t = 0	t = 6	*P**	t = 0	t = 6	*P**
Insulin (pmol/L)	Control	38 ± 6	29 ± 6	<.01	63 ± 10	56 ± 4	<.01
	Acipimox	35 ± 4	18 ± 3		64 ± 14	39 ± 6	
C-peptide (pmol/L)	Control	373 ± 74	232 ± 45	<.05	598 ± 70	483 ± 64	<.01
	Acipimox	367 ± 64	120 ± 35		713 ± 94	330 ± 57	
Glucagon (ng/L)	Control	54 ± 6	54 ± 5	.02	73 ± 14	72 ± 10	NS
	Acipimox	61 ± 11	84 ± 13		72 ± 10	81 ± 9	
Cortisol (nmol/L)	Control	400 ± 67	217 ± 27	NS	200 ± 19	158 ± 22	NS
	Acipimox	302 ± 26	303 ± 59		212 ± 36	297 ± 28	
Epinephrine (nmol/L)	Control	0.16 ± 0.04	0.25 ± 0.03	.02	0.14 ± 0.04	0.12 ± 0.03	<.01
	Acipimox	0.16 ± 0.07	0.77 ± 0.22		0.27 ± 0.08	0.54 ± 0.10	
Norepinephrine (nmol/L)	Control	1.13 ± 0.16	1.03 ± 0.25	NS	1.63 ± 0.21	1.86 ± 0.25	NS
	Acipimox	0.82 ± 0.19	1.01 ± 0.15		1.50 ± 0.17	1.89 ± 0.34	

**P* value of difference between curves of control and acipimox studies over time.

tions and imperfections have been addressed by Hellerstein et al.[6-8,12]

We have previously shown that MIDA yields consistently lower rates of gluconeogenesis than those measured with ^2H_2O.[13] However, the direction of the changes and the absolute changes of gluconeogenic flux over time are not different between both techniques. In the present study gluconeogenesis contributed approximately 25% to endogenous glucose production in both lean and obese groups after 16 hours of fasting, which is comparable to previous findings using [2-^{13}C]glycerol MIDA.[6,13-15] Between 16 and 22 hours of fasting the contribution of GNG to endogenous glucose production increased from 25% to 36% and from 25% to 42% in the lean and obese control groups, respectively. This increase is in agreement with studies performed with the deuterated water technique.[16,17]

A potential confounding variable in our study is the age difference between the obese and the lean subjects. Although age has effects on glucose homeostasis, these effects mainly concern peripheral glucose metabolism.[18] Euglycemic clamp studies have shown that hepatic glucose production and hepatic sensitivity to insulin remain unaltered with advancing age.[19,20] Therefore, the age difference between the lean and obese males is unlikely to have influenced the interpretation of our results.

Our data suggest that in lean males acute lowering of plasma FFA levels does not significantly affect endogenous glucose production in the postabsorptive state. This result is in accordance with the study of Chen et al,[3] who showed that lowering of plasma FFA with nicotinic acid during a 16- to 20-hour fast in healthy volunteers had no effect on glucose production. However, in obese males FFA suppression decreased glucose production to a lesser degree than physiological FFA levels, suggesting that physiological FFA levels partially inhibit glucose production. Boden et al[16] found similar results obtained with nicotinic acid administration to overweight volunteers.

In lean males, absolute gluconeogenesis showed no difference between the acipimox and control studies during 16 and 22 hours of fasting due to increased gluconeogenesis in the last 2 hours of the acipimox study, probably caused by an increase in epinephrine levels during the last 2 hours. The data between 16 and 20 hours of fasting are in agreement with literature, as in lean males gluconeogenesis was higher in the control study than in the acipimox study.[3,16] The fact that these other studies did not find a contra-regulatory effect of epinephrine are probably due to the fact that these studies lasted 2 hours shorter than our study, therefore obscuring the effect of nicotinic acid on epinephrine secretion.

The differences in insulin and glucagon concentrations induced by acipimox administration are not thought to have influenced the results since differences in glycogen metabolism between acipimox and control studies were evident before differences in insulin and glucagon concentrations appeared. Furthermore, the differences in plasma insulin and glucagon between acipimox and control studies were small and similar in lean and obese subjects, unlike studies specially designed to investigate effects of these hormones on glucose metabolism.[21,22]

The results show that FFA contribute to the retention of hepatic glycogen content during short term fasting in obese but not in lean males and are consistent with a study performed by Muller et al in which liver biopsies revealed a higher glycogen concentration in obese than in lean subjects (515 *v* 308 mg/g protein) after a 13 hour fast.[1] Contrary to our results in lean males, Stingl et al, using ^{13}C NMR spectroscopy, found that in lean males hepatic glycogen concentration declined during fasting, and that high levels of FFA by infusing lipid/heparin restrained liver glycogen breakdown.[4] Differences in study design might explain the different results. They studied the effect of supraphysiological concentrations (~2.2 mmol/L) of FFA, whereas our study focused on the influence of low-normal (physiological) plasma FFA levels.

Our finding that FFA suppressed absolute glycogenolysis in both lean and obese males is consistent with results found by others.[3,23]

Intrahepatic gluconeogenic flux can be directed towards plasma (direct gluconeogenesis) or toward glycogen (indirect gluconeogenesis). In lean males, direct and indirect gluconeogenesis were unaffected by physiological FFA levels. These results in the lean control group are in accordance to literature; Hellerstein et al compared 11 hours to 60 hours of fasting in healthy volunteers under basal conditions using the same tech-

nique as we used in this study. They showed that the direct pathway was quantitatively the most important component of gluconeogenic flux and that the indirect pathway was unaffected by fasting.[6] In obese males, however, we showed that both direct and indirect gluconeogenesis were stimulated by FFA. This effect of FFA on the 2 pathways of gluconeogenesis in obese males is a novel finding, as we are not aware of comparable studies in obese subjects.

The biochemical mechanism by which FFA promote the retention of hepatic glycogen content in obese but not in lean subjects remains to be elucidated. Despite differences in glycogen metabolism in lean and obese subjects, plasma FFA levels in lean and obese subjects showed remarkable similarity, suggesting that FFA are not solely responsible for the increased retention of glycogen in obesity. Therefore, we propose that the higher insulin levels found in obese subjects could have potentiated the effect of FFA on glycogenolysis and glycogen synthesis retained as glycogen, for both FFA and insulin are known to inhibit glycogen breakdown.[3,22]

In conclusion, physiological FFA levels have different effects on intrahepatic glucose pathways in lean and obese subjects. In obese subjects physiological FFA levels inhibit glucose production and glycogenolysis, while stimulating gluconeogenesis and glycogen synthesis retained as glycogen in obese subjects. In lean subjects physiological FFA levels only inhibit glycogenolysis. Physiological plasma FFA levels contribute to the retention of hepatic glycogen in obesity, whereas this effect is absent in lean subjects.

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REFERENCES

1. Muller C, Assimacopoulos-Jeannet F, Mosimann F, et al: Endogenous glucose production, gluconeogenesis and liver glycogen concentration in obese non-diabetic patients. Diabetologia 40:463-468, 1997

2. Llado I, Palou A, Pons A: Hepatic glycogen and lactate handling in dietary obese rats. Ann Nutr Metab 42:181-188, 1998

3. Chen X, Iqbal N, Boden G: The effects of free fatty acids on gluconeogenesis and glycogenolysis in normal subjects. J Clin Invest 103:365-372, 1999

4. Stingl H, Krssak M, Krebs M, et al: Lipid-dependent control of hepatic glycogen stores in healthy humans. Diabetologia 44:48-54, 2001

5. Lam TK, Carpentier A, Lewis GF, et al: Mechanisms of the free fatty acid-induced increase in hepatic glucose production. Am J Physiol Endocrinol Metab 284:E863-E873, 2003

6. Hellerstein MK, Neese RA, Linfoot P, et al: Hepatic gluconeogenic fluxes and glycogen turnover during fasting in humans. A stable isotope study. J Clin Invest 100:1305-1319, 1997

7. Hellerstein MK, Neese RA: Mass isotopomer distribution analysis: A technique for measuring biosynthesis and turnover of polymers. Am J Physiol 263:E988-1001, 1992

8. Hellerstein MK, Neese RA, Letscher A, et al: Hepatic glucose-6-phosphatase flux and glucose phosphorylation, cycling, irreversible disposal, and net balance in vivo in rats. Measurement using the secreted glucuronate technique. Metabolism 46:1390-1398, 1997

9. Steele R: Influences of glucose loading and of injected insulin on hepatic glucose output. Ann NY Acad Sci 82:420-430, 1959

10. Wolfe RR: Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis. New York, NY, Wiley, 1992

11. Neese RA, Schwarz JM, Faix D, et al: Gluconeogenesis and intrahepatic triose phosphate flux in response to fasting or substrate loads. Application of the mass isotopomer distribution analysis technique with testing of assumptions and potential problems. J Biol Chem 270:14452-14466, 1995

12. Landau BR: Problems with the assumed biochemical basis for estimating hepatic glycogen turnover using glucuronide formation and labeled galactose. Metabolism 49:1374-1378, 2000

13. Ackermans MT, Pereira Arias AM, Bisschop PH, et al: The quantification of gluconeogenesis in healthy men by (2)H(2)O and 2-(13)C glycerol yields different results: Rates of gluconeogenesis with (2)H(2)O are higher than those measured with 2-(13)C glycerol. J Clin Endocrinol Metab 86:2220-2226, 2001

14. Trimmer JK, Schwarz JM, Casazza GA, et al: Measurement of gluconeogenesis in exercising men by mass isotopomer distribution analysis. J Appl Physiol 93:233-241, 2002

15. Christiansen MP, Linfoot PA, Neese RA, et al: Effect of dietary energy restriction on glucose production and substrate utilization in type 2 diabetes. Diabetes 49:1691-1699, 2000

16. Boden G, Chen X, Capulong E, et al: Effects of free fatty acids on gluconeogenesis and autoregulation of glucose production in type 2 diabetes. Diabetes 50:810-816, 2001

17. Wajngot A, Chandramouli V, Schumann WC, et al: Quantitative contributions of gluconeogenesis to glucose production during fasting in type 2 diabetes mellitus. Metabolism 50:47-52, 2001

18. Elahi D, Muller DC: Carbohydrate metabolism in the elderly. Eur J Clin Nutr 54:S112-S120, 2000 (suppl 3)

19. Meneilly GS, Minaker KL, Elahi D, et al: Insulin action in aging man: Evidence for tissue-specific differences at low physiologic insulin levels. J Gerontol 42:196-201, 1987

20. DeFronzo RA: Glucose intolerance and aging: Evidence for tissue insensitivity to insulin. Diabetes 28:1095-1101, 1979

21. Dobbins RL, Davis SN, Neal D, et al: Rates of glucagon activation and deactivation of hepatic glucose production in conscious dogs. Metabolism 47:135-142, 1998

22. Edgerton DS, Cardin S, Emshwiller M, et al: Small increases in insulin inhibit hepatic glucose production solely caused by an effect on glycogen metabolism. Diabetes 50:1872-1882, 2001

23. Roden M, Stingl H, Chandramouli V, et al: Effects of free fatty acid elevation on postabsorptive endogenous glucose production and gluconeogenesis in humans. Diabetes 49:701-707, 2000