Premenopausal Advantages in Postprandial Lipid Metabolism Are Lost in Women With Type 2 Diabetes

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OBJECTIVE — Women with type 2 diabetes appear to lose the protection against cardiovascular disease afforded by estrogens. We examined the effects of menopausal status on postprandial clearance of dietary fat in healthy and diabetic women.

RESEARCH DESIGN AND METHODS — Fasting subjects (premenopausal and postmenopausal control subjects, premenopausal and postmenopausal diabetic women, all $n = 8$) were given a meal containing the stable isotope 1,1,1-^{13}C-tripalmitin, with blood and breath sampled for 6 and 24 h, respectively, in the postprandial period. Lower levels of ^{13}C-palmitic acid (^{13}C-PA) in the triglyceride fraction implies more efficient chylomicron clearance, lower levels of ^{13}C-PA in the nonesterified fatty acid (NEFA) fraction implies improved dietary NEFA entrapment, and higher levels of $^{13}CO_2$ in the breath denote more efficient of oxidation of dietary-derived lipid.

RESULTS — In diabetic women, there were no differences between the pre- and postmenopausal groups for any of these parameters. In contrast, premenopausal control subjects, compared with postmenopausal control subjects, had lower ^{13}C-PA in the triglyceride fraction area under the curve (AUC) (premenopausal median [range] 25.2 [12.1–49.4 mmol/l] per 6 h, postmenopausal 48.5 [15.5–77.2 mmol/l] per 6 h; $P < 0.01$) and higher $^{13}CO_2$ levels in the breath AUC (premenopausal 22.5 [18.0–31.5%] of administered dose, postmenopausal 17.2 [11.2–31.5%] of administered dose; $P < 0.01$) with no difference between groups in levels of ^{13}C-PA in the NEFA fraction AUC.

CONCLUSIONS — The premenopausal advantage in clearance of dietary lipid is not seen in premenopausal diabetic women. This is likely to promote an atherogenic lipoprotein profile and may contribute to the loss of cardiovascular disease protection seen in diabetic women.

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Patients with type 2 diabetes have an increased cardiovascular risk, which is particularly marked in diabetic women (1). In the nondiabetic population, women are relatively protected against coronary heart disease (CHD) when compared with men, an effect attributed to the physiological effects of estrogens (2,3). In contrast, diabetic women have an equivalent rate of CHD to diabetic men, appearing to lose the protection of estrogens (1).

There are many contender mechanisms to account for the high CHD risk observed in diabetes (4), including diabetic dyslipidemia, which is thought to be particularly atherogenic and is characterized by fasting hypertriglyceridemia, low HDL cholesterol, and small dense LDL (4). The impact of the postprandial triglyceride load will be underestimated by examining fasting triglyceride values, as the typical western diet and pattern of feeding usually results in maintenance of the postprandial state for ~16 h each day (5). Postprandial triglyceride levels are determined by the metabolism of chylomicrons and VLDL. Chylomicron-triglyceride is hydrolyzed by the action of the enzyme lipoprotein lipase (LPL) releasing nonesterified fatty acids (NEFAs), the remaining chylomicron remnants being taken up by the liver via LDL receptors (6). Premenopausal nondiabetic women have been shown to have lower postprandial triglyceride levels than postmenopausal women (7,8), probably due to upregulation of hepatic LDL receptor by estrogens (9), thus increasing chylomicron remnant clearance. However, reduced chylomicron clearance is a feature of diabetes (10).

We hypothesized that impaired clearance of circulating triglyceride-rich lipoproteins (TRLs) in diabetic women would impair the ability of increased estrogen-mediated clearance at the liver to ameliorate postprandial hypertriglyceridemia. The estrogen-associated benefits on postprandial hypertriglyceridemia observed in healthy control subjects (7,8) would thus not be present in women with type 2 diabetes, and the potential benefit in reducing atherosclerotic risk may be lost. We studied the extent to which perturbations in postprandial triglyceride clearance and fatty acid uptake in the periphery occur in premenopausal and postmenopausal women with and without diabetes.

RESEARCH DESIGN AND METHODS

— Ethical approval for the study was obtained from the local ethics

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Abbreviations: AUC, area under the curve; CHD, coronary heart disease; IRMS, isotope ratio mass spectrometry; LPL, lipoprotein lipase; NEFA, nonesterified fatty acid; TRL, triglyceride-rich lipoprotein; ^{13}C-PA, ^{13}C-palmitic acid.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

committee, and all subjects gave informed consent. Women with type 2 diabetes were recruited from a hospital clinic and age-matched healthy control subjects from hospital staff and volunteers. Four groups were recruited: premenopausal women with diabetes, postmenopausal women with diabetes (these groups matched for duration of diabetes), premenopausal healthy control subjects, and postmenopausal healthy control subjects. Postmenopausal status was confirmed by amenorrhea for greater than a year and elevated leutinizing hormone and follicle-stimulating hormone levels. Subjects taking either the oral contraceptive pill or estrogen hormone replacement therapy or taking lipid-lowering therapy were excluded.

Subjects were asked to avoid foods naturally enriched with ^{13}C (e.g., maize products and cane sugar) and avoid strenuous exercise and alcohol for 2 days before the study. They were provided with a standard evening meal before the study day to replicate the fasting state, after which they drank only water until the study commenced. Subjects were asked to refrain from taking any medication (including oral hypoglycemic agents) from the evening before the study.

On the study day, subjects attended our unit at 0800. Height and weight were measured with fat mass and lean body mass measured by bioelectrical impedance (BodyStat, Isle of Man, U.K.). Resting energy expenditure and substrate oxidation were determined by indirect calorimetry (Gas Exchange Monitor, Europa Scientific, Crewe, UK), and a specimen of expired air was collected to measure baseline ^{13}C-excretion on breath. An indwelling cannula was sited in a forearm vein, and a fasting blood sample was withdrawn. The subjects consumed a standard 3,720-kJ test meal of breakfast cereal and milk, bread, margarine, and cheese together with a lipid:glucose:casein emulsion containing the tracer (700 mg [1,1,1-^{13}C]tripalmitin 99 atom percent excess; Masstrace, Woburn, MA), which provided 45 g lipid, 93 g carbohydrate, and 33 g protein (11). Thereafter, venous blood samples were collected at one-half-hourly intervals for the first 3 h and then hourly until 6 h after label administration when the study was completed. The patients remained seated or supine and under constant supervision for the duration of the study period. No additional foods or liquids, except for water, were permitted during the study. Specimens of expired air were collected and indirect calorimetry performed hourly for the 6 h of the study, and the subjects were instructed to collect four further samples at home until 24 h after the labeled meal.

Measurement of ^{13}C enrichment in breath and lipid fractions from plasma

[^{13}C]-Enrichment of CO_2 excreted on breath was measured by continuous-flow isotope ratio mass spectrometry (IRMS) using a 20/20 stable isotope analyzer equipped with a gas/solid/liquid interface (PDZ-Europa, Crewe, UK) (12). The proportion of administered labeled palmitic acid converted to $^{13}CO_2$ over 24 h was calculated from the [^{13}C]-enrichment and whole-body CO_2 excretion.

Total lipids were isolated from plasma (1 ml) by extraction with chloroform/methanol (13) using triheptadecanoin and heneicosanoic acid as internal standards. Triglyceride and NEFA were purified by solid phase extraction using 100-mg aminopropylsilica cartridges, and fatty acid methyl esters were prepared by incubation with methanol containing 2% (vol/vol) H_2SO_4 for 18 h at 50°C (12). [^{13}C] enrichment in palmitic acid was determined by gas chromatography–combustion IRMS (14). Fatty acid methyl esters were resolved using an Hewlett Packard 6890 gas chromatography equipped with a 50 m × 0.25 μm × 0.32 mm BPX-70 fused silica capillary column (SGE Europe Limited, Milton Keynes, UK). Fatty acid methyl esters were converted to CO_2 by heating at 860°C in the presence of PtCuO using an Orchid combustion interface (PDZ-Europa). $^{13}CO_2$:$^{12}CO_2$ ratio was determined by a 20/20 Stable Isotope Analyzer (PDZ-Europa).

Plasma triglyceride, NEFA, glucose, insulin, and gonadotrophin measurements

Plasma triglyceride and NEFA concentrations were calculated from the gas chromatography–combustion IRMS chromatograms using the peak area for the fatty acids and standards within each triglyceride and NEFA fraction, respectively. Plasma glucose concentration was determined using an automated glucose analyzer (AU600, Olympic Diagnostics, Southall, UK), and plasma insulin concentration by an automated enzyme-linked immunosorbent assay (ES700: Roche Diagnostics, Lewes, UK). HDL cholesterol and total cholesterol measurements were made from stored plasma using enzymatic methods with commercial assay kits (Sigma, Poole, UK). Circulating leutinizing hormone and follicle-stimulating hormone levels were analyzed using a two-site immunochemiluminometric assay (Bayer, Newbury, UK).

Metabolic model and data analysis

The primary changes of interest were the kinetics and time course of ^{13}C-palmitic acid (^{13}C-PA) in the triglyceride and NEFA pools within the circulation and the kinetics and time course of ^{13}C label excretion in breath. A summary statement of the triglyceride and NEFA excursions (both labeled and unlabelled) was derived by determining the area under the curve (AUC) (15). Prolonged retention of ^{13}C-PA as triglyceride in the circulation was assumed to reflect impaired chylomicron clearance from the circulation by peripheral tissues. Elevation of ^{13}C-PA in the NEFA fraction reflected hydrolysis of chylomicron triglyceride and impaired entrapment of the resultant fatty acids by peripheral tissues. The extent of recovery of ^{13}C in breath as $^{13}CO_2$ reflects oxidation of dietary lipid by peripheral tissues, particularly skeletal muscle.

Initial power calculations (Sample Power version 2.0; SPSS) indicated that a sample size of eight would have an 80% power to detect a 25% difference in the primary outcome variables, namely lipid clearance (triglyceride AUC and ^{13}C-PA in the triglyceride fraction AUC), entrapment (NEFA AUC and ^{13}C-PA in the NEFA fraction AUC), and cumulative oxidation ($^{13}CO_2$ on breath). Data were analyzed using a statistical computer package (SPSS version 10.0). Given that the numbers of subjects in each group were relatively small and that much of the data were not normally distributed, the results are presented as the median (range). Statistical significance of differences between groups was determined by Kruskal-Wallis ANOVA, and associations between variables were examined using Spearman's rank correlation. Significance was assumed where $P < 0.05$.

RESULTS — Subject characteristics are shown in Table 1. There were eight subjects in each group. In the premeno-

pausal diabetes group, two subjects were diet controlled, two were taking metformin alone, and four were taking a sulfonylurea in combination with metformin. In the postmenopausal diabetes group, one subject was diet controlled, three were taking metformin alone, and four were taking a sulfonylurea in combination with metformin. Although the postmenopausal women with and without diabetes were of comparable weight and BMI, the premenopausal diabetic women were significantly heavier and had a higher BMI than their counterparts without diabetes ($P < 0.01$) (Table 1). There were no differences between the diabetic groups in glycemic control as estimated by fasting plasma glucose or HbA_{1c}. Apart from significantly lower plasma NEFA values in the postmenopausal women without diabetes, there were no significant differences associated with menopausal status in either the diabetic or control women. Both the premenopausal and postmenopausal diabetic groups had higher fasting glucose, triglyceride, and NEFA values, together with lower fasting HDL cholesterol levels than their nondiabetic counterparts (all $P < 0.01$, except for postmenopausal triglyceride where $P < 0.05$).

The summative statements for the glucose, insulin, labeled triglyceride, and NEFA responses over the postprandial period are shown in Table 2 and the changes in total and labeled lipid concentration over time shown in Fig. 1.

In the postprandial period, total triglyceride rose from the baseline level in all the groups (Fig. 1*A*) and did not return to baseline by the end of the study. Postprandial triglyceride levels were higher in the diabetic groups, as demonstrated by higher triglyceride AUC compared with healthy control subjects (Table 2). The rise in the premenopausal control subjects was less than that seen in the postmenopausal control subjects (Fig. 1*A*), and premenopausal control subjects had lower postprandial triglyceride AUC than their postmenopausal counterparts (Table 2). In contrast, postprandial triglyceride levels remained similar in the premenopausal and postmenopausal diabetic groups (Fig. 1*A*) with no significant difference in postprandial triglyceride AUC between premenopausal and postmenopausal women with diabetes (Table 2).

Postprandial ^{13}C-PA levels in the triglyceride fraction rose in all groups and did not return to baseline at the end of the

Table 1—*Subject characteristics of pre- and postmenopausal women with and without diabetes*

	Women without diabetes		Women with diabetes		Significant difference + diabetes	
	Premenopausal	Postmenopausal	Premenopausal	Postmenopausal	Premenopausal	Postmenopausal
n	8	8	8	8		
Age (years)	42 (34–56)*	58 (46–68)	39 (32–54)*	61 (53–70)	NS	NS
Weight (kg)	64.6 (52.7–121.9)	77.7 (56.8–96.7)	102.8 (59.8–123.1)	82.8 (76.9–105.8)	0.01	NS
BMI (kg/m^2)	26.5 (21.7–42.2)	30 (21.9–37.3)	37.8 (23.1–46.3)	32.6 (27.9–35.2)	0.01	NS
HbA_{1c} (%)	ND	ND	9.1 (6.8–14.8)	8 (6.0–9.1)	—	—
Fasting values						
Glucose (mmol/l)	5.4 (5.2–6.1)	5.3 (4.6–6.1)	9.9 (5.6–14.0)	10.5 (7.5–17.9)	0.01	0.05
Insulin	10.7 (4.8–12.3)	7.9 (5–34.3)	12.8 (4.5–28.5)	13.6 (9.1–31.3)	NS	NS
Triglycerides (mmol/l)	0.9 (0.7–2.2)†	1.6 (0.5–2.7)	2.2 (1.7–3.0)	2.4 (1.6–5.8)	0.01	0.05
NEFA (mmol/l)	163 (75.3–198.5)†	113.9 (84.2–141.9)	189.5 (169.4–244.1)	194 (129.1–327.4)	0.01	0.01
Total cholesterol (mmol/l)	4.7 (3.8–6.9)*	6.6 (4.8–9.3)	4.8 (3.6–6.6)	5.1 (4.0–6.8)	NS	0.01
HDL cholesterol (mmol/l)	1.8 (1.1–2.3)	1.5 (1.1–2.2)	1.0 (0.7–1.5)	1.1 (0.8–1.3)	0.01	0.01

Values shown are median (range). Superscript denotes significantly different from postmenopausal women within corresponding group by Kruskal-Wallis ANOVA: *$P < 0.01$; †$P < 0.05$. ND, HbA_{1c} not determined in women without diabetes; NS, not significant. The significance of differences between women with and without diabetes for the corresponding menopausal status is also shown.

Table 2—*Summative statements as AUC over the study period of pre- and postmenopausal women with and without diabetes*

	Women without diabetes		Women with diabetes		Significant difference ± diabetes	
	Premenopausal	Postmenopausal	Premenopausal	Postmenopausal	Premenopausal	Postmenopausal
n	8	8	8	8		
Glucose AUC (mmol/l per 6 h)	78.9 (67.5–230.1)	73.7 (65.4–249.6)	166.8 (100.4–219.0)	118.4 (57.7–236.4)	NS	NS
Insulin AUC (pmol/l per 6 h)	600.7 (438.7–968.9)	541.8 (148.3–1,199.1)	479.6 (170.1–1,749.3)	509.7 (240.4–728.0)	NS	NS
Triglycerides AUC (mmol/l per 6 h)	7.0 (4.3–14.6)*	12.4 (4.7–18.5)	21.1 (15.5–41.8)	18.4 (11.5–40.8)	0.01	0.05
^{13}C-PA in triglycerides AUC (ng/ml per 6 h)	25.2 (12.1–49.4)†	48.5 (15.5–77.2)	53.8 (27.7–172.0)	47.8 (24.6–121.6)	0.01	NS
NEFA AUC (mmol/l per 6 h)	462.9 (368.6–699.3)	372.0 (282.2–555.2)	569.7 (424.9–791.5)	670.2 (550.0–1,252.4)	0.05	0.01
^{13}C-PA in NEFA AUC (ng/ml per 6 h)	2.2 (1.8–2.9)	2.1 (1.2–5.2)	1.7 (1.5–5.8)	2.2 (1.4–6.5)	NS	NS
Breath $^{13}CO_2$ AUC (% of administered dose per 24 h)	22.5 (18.0–31.5)†	17.2 (11.2–23.02)	24.1 (15.4–29.1)	22.5 (19.1–34.9)	NS	0.01

Values shown are median (range). Superscript denotes significantly different from postmenopausal women within corresponding group by Kruskal-Wallis ANOVA: *$P < 0.05$; †$P < 0.01$. The significance of differences between women with and without diabetes for the corresponding menopausal status is also shown.

study period. Diabetic women had higher ^{13}C-PA in the triglyceride fraction AUC than control women (Table 2), which persisted when adjusted for BMI. Premenopausal control subjects had lower ^{13}C-PA in the triglyceride fraction AUC than the postmenopausal control group. Menopausal status did not affect the ^{13}C-PA in the triglyceride fraction AUC in the diabetes groups (Fig. 1*B*, Table 2).

Circulating NEFA levels fell from baseline in all groups, returning back to this value at the end of the study (Fig. 1*C*). There was no effect of menopausal status on the NEFA AUC in either the diabetic or control women, although higher NEFA AUC were observed in the diabetic postmenopausal women than in the healthy control subjects ($P < 0.01$). There were no significant differences in levels of ^{13}C-PA in the NEFA fraction AUC in any of the groups (Fig. 1*D*, Table 2).

In all of the groups, breath excretion of $^{13}CO_2$ increased until 3 h after ingestion of the labeled meal and then reached a plateau for 3 h before falling (Fig. 1*E*). Premenopausal control women exhibited higher recovery of label on breath than their postmenopausal counterparts ($P < 0.01$), whereas no differences were observed between the subgroups of diabetic women. The postmenopausal control women had lower breath recovery values than their diabetic counterparts ($P < 0.01$).

CONCLUSIONS — In this study, we examined chylomicron metabolism and the exogenous pathway of lipoprotein metabolism, contrasting premenopausal and postmenopausal women with and without diabetes using a stable isotope technique to follow the fate of dietary lipids. The addition of the stable isotope label allowed the fate of the dietary triglyceride to be assessed without interference from VLDL. Taken together, these results suggest that premenopausal healthy women appear to be better able to clear dietary lipid from their circulation than their postmenopausal counterparts. We have been unable to demonstrate the same beneficial effect of premenopausal status women with diabetes.

When considering the healthy control subjects, premenopausal status is associated with improved triglyceride clearance, including both total and dietary-derived triglyceride, supporting previous studies (7,8). The impaired

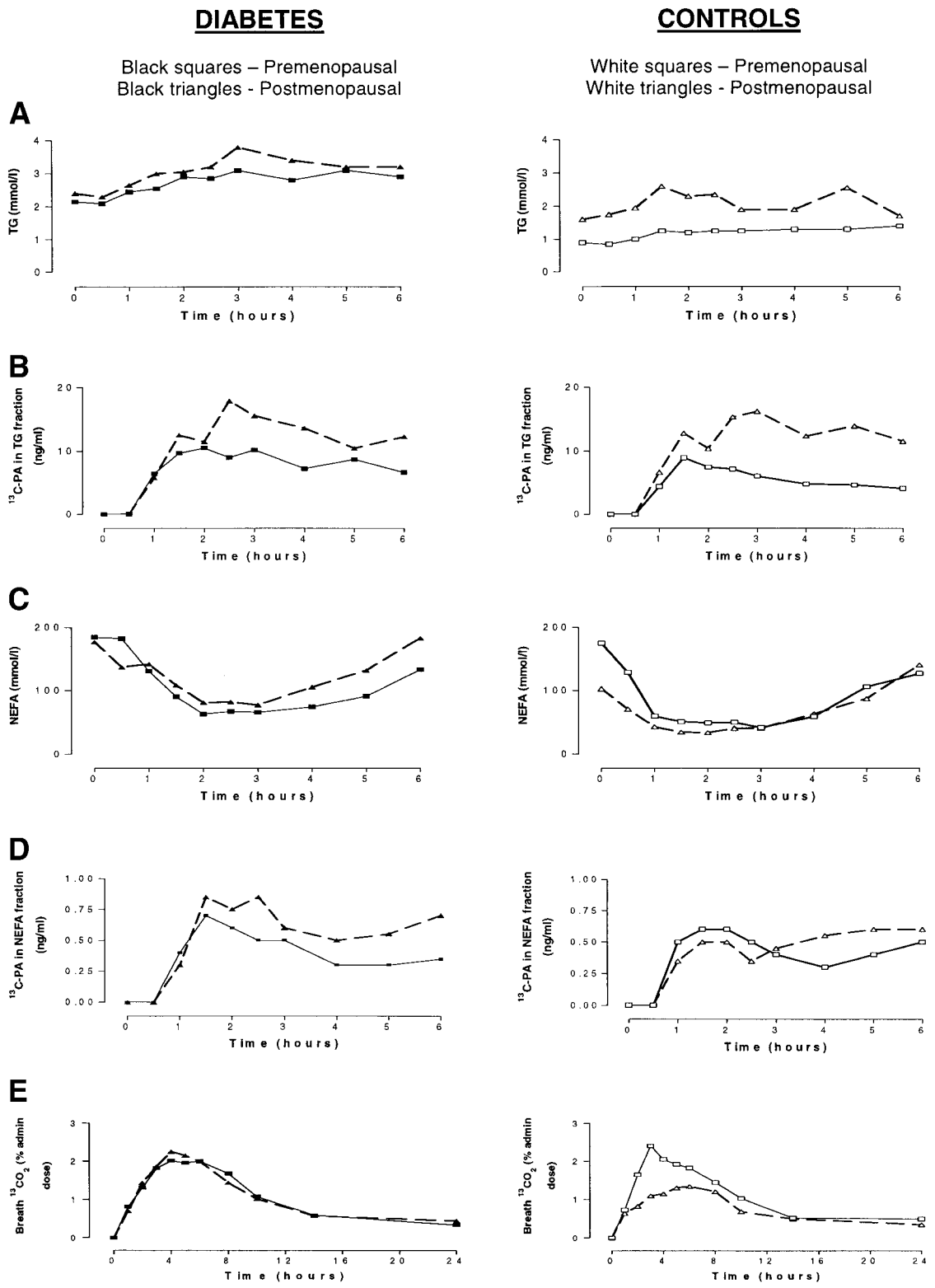

Figure 1—*Postprandial lipid metabolism in pre- and postmenopausal women with and without type 2 diabetes.* A*: Total triglycerides (TG).* B*: ^{13}C-PA in triglyceride fraction.* C*: Total NEFAs.* D*: ^{13}C-PA in NEFA fraction.* E*: Breath $^{13}CO_2$ excretion (all values are medians).*

clearance of ^{13}C-PA–labeled triglyceride from the circulation supports impaired clearance of dietary triglyceride, which could be due to either reduced hydrolysis of chylomicron triglyceride by LPL as a result of reduced LPL activity or competition for LPL binding with VLDL or reduced hepatic clearance of chylomicrons and their remnants by LDL receptor (8).

Evidence from the literature is conflicting, and it is not possible to attribute these effects to one of these mechanisms. Animal studies support both increased and reduced LPL activity due to the presence of estrogen (16,17), but human studies suggest no effect (18,19). In contrast, there is ample evidence in favor of increased LDL receptor activity (9). An alternative pathway for chylomicron clearance has been proposed via the LDL receptor–related protein, although animal studies suggest that hepatic LDL receptor–related protein expression is reduced in the presence of estrogen (20). In our diabetic women, no difference was seen in triglyceride clearance, with premenopausal diabetic women exhibiting similar total or dietary-derived triglyceride levels to those of the postmenopausal diabetic women. It is possible that in diabetes either LDL receptor does not respond to estrogen or it is already maximally upregulated by a chronic demand for chylomicron remnant clearance.

We were able to follow the production of labeled NEFA and found no differences in any of the groups. Considering $^{13}CO_2$ production in the healthy control subjects, this was greater in the premenopausal than the postmenopausal women, suggesting increased NEFA tissue oxidation. There are confounding possibilities to account for this apart from menopausal status (e.g., age, physical fitness). However, our observation is supported by previous studies that have demonstrated a stimulatory effect of estrogen on lipid oxidation (21,22).

NEFA oxidation was higher in the diabetic subjects than the control subjects, but no effect was seen due to menopausal status. This is initially surprising as previous studies show reduced oxidation in diabetes (23). However, NEFA oxidation is strongly affected by substrate level, which was much higher in our diabetic subjects but normalized in other studies.

There have been no other studies looking at the effects of endogenous estrogens (i.e., menopausal status) on lipid metabolism in women with type2 diabetes. Friday et al. (24) have previously demonstrated that oral estrogen replacement therapy results in a small reduction in increments in postprandial triglyceride levels when given to postmenopausal diabetic women, although no change in absolute triglyceride levels was seen.

Premenopausal women have lower LDL cholesterol and higher HDL cholesterol (25), probably due to increased LDL receptor activity stimulated by estrogens (9). Postprandial hypertriglyceridemia due to impaired chylomicron clearance will have important effects on LDL and HDL metabolism. In the presence of high circulating levels of triglyceride, in either chylomicrons or VLDL (TRL), the process of neutral lipid exchange results in transfer of triglyceride from TRLs to HDL and LDL with cholesteryl esters being transferred the other way back to the TRLs (6). The triglyceride-rich HDL and LDL molecules are further metabolized in the liver because of a higher affinity for hepatic lipase, resulting in the catabolism of HDL and the formation of small dense LDL. The resultant lipoprotein profile of small dense LDL, low HDL cholesterol levels, and hypertriglyceridemia is typical of type 2 diabetes and is recognized to be particularly atherogenic (26).

We accept that there are limitations to our study, given the relatively small numbers of subjects in each group and the possible compounding influence of factors such as differences in age, body composition, and lifestyle. The numbers studied using this detailed technique are by necessity small, although they are supported by the power calculation reflecting the large differences in postprandial lipid metabolism even allowing for the large variance. Although there were no statistical differences between the premenopausal and postmenopausal diabetic women in glycemic control or BMI, the premenopausal women tended to have higher HbA_{1c} levels, but their metabolism was better balanced at the time of the study as shown by a tendency toward lower fasting glucose. There was a trend toward higher BMI in the premenopausal diabetic women, which may have contributed to the lack of difference seen in comparison with the postmenopausal diabetic group. However, this effect may also contribute to the epidemiological observations of increased CHD risk in diabetic women, considering that the majority of patients with type 2 diabetes are overweight. It is difficult to control for age in studies of menopausal status, but these effects of age would operate in both control and diabetic women.

We postulated that the impact of the impaired clearance of chylomicron remnants in type 2 diabetes will result in the swamping of the ability of the hepatic LDL receptor to clear remnants even when augmented by estrogens. Our study confirms impaired postprandial triglyceride clearance in diabetes with our diabetic subjects all exhibiting impaired clearance when compared with the healthy control subjects whatever their menopausal status. In our control group, however, postprandial metabolism was more effective in the premenopausal women. This is attributed to the effects of estrogens, although the effects of other hormonal differences (such as androgens or progesterone) could have a role. Our hypothesis that the differences in postprandial metabolism are attributable to estrogen are supported by studies showing that exogenous estrogens do improve chylomicron clearance (9,18,27). The absolute levels of triglyceride are higher in the diabetic women when compared with the healthy control subjects, and this is likely to generate a proatherogenic lipoprotein profile. The improvement in clearance in the healthy women would be expected to reduce the atherosclerotic risk in healthy premenopausal women, which is a benefit not seen in our diabetic patients. However, both lifestyle (28,29) and pharmacological (30,31) intervention may improve chylomicron clearance, so women with diabetes may particularly benefit from such measures.

In summary, we have demonstrated that healthy premenopausal women have lower postprandial triglyceride levels compared with healthy postmenopausal women due to improved clearance of chylomicron remnants. However, this premenopausal advantage is lost in women with type 2 diabetes, which may help to explain the high risk of CHD in this group and the apparent disadvantage for diabetic women with respect to CHD.

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