Normal Postprandial Nonesterified Fatty Acid Uptake in Muscles Despite Increased Circulating Fatty Acids in Type 2 Diabetes

Sébastien M. Labbé,1 Etienne Croteau,2 Thomas Grenier-Larouche,1 Frédérique Frisch,1 René Ouellet,2 Réjean Langlois,2 Brigitte Guérin,2 Eric E. Turcotte,2 and André C. Carpentier1

OBJECTIVE—Postprandial plasma nonesterified fatty acid (NEFA) appearance is increased in type 2 diabetes. Our objective was to determine whether skeletal muscle uptake of plasma NEFA is abnormal during the postprandial state in type 2 diabetes.

RESEARCH DESIGN AND METHODS—Thigh muscle blood flow and oxidative metabolism indexes and NEFA uptake were determined using positron emission tomography coupled with computed tomography (PET/CT) with [1-¹⁴C]acetate and [14(R,S)-¹⁸F]fluoro-6-thia-heptadecanoic acid ([¹⁸FTHA) in seven healthy control subjects (CON) and seven subjects with type 2 diabetes during continuous oral intake of a liquid meal to achieve steady postprandial NEFA levels with insulin infusion to maintain similar plasma glucose levels in both groups.

RESULTS—In the postprandial state, plasma NEFA level was higher in type 2 diabetic subjects versus CON (P < 0.01), whereas plasma glucose was at the same level in both groups. Muscle NEFA fractional extraction and blood flow index levels were 56% (P < 0.05) and 24% (P = 0.16) lower in type 2 diabetes, respectively. However, muscle NEFA uptake was similar to that of CON (quadriceps femoris [QF] 1.47 ± 0.23 vs. 1.37 ± 0.24 nmol·g⁻¹·min⁻¹, P = 0.77; biceps femoris [BF] 1.54 ± 0.26 vs. 1.46 ± 0.28 nmol·g⁻¹·min⁻¹, P = 0.85). Muscle oxidative metabolism was similar in both groups. Muscle NEFA fractional extraction and blood flow index were strongly and positively correlated (r = 0.79, P < 0.005).

CONCLUSIONS—Postprandial muscle NEFA uptake is normal despite elevated systemic NEFA levels and acute normalization of plasma glucose in type 2 diabetes. Lower postprandial muscle blood flow with resulting reduction in muscle NEFA fractional extraction may explain this phenomenon.

Increased intramyocellular triglyceride (IMTG) level correlates with impaired insulin sensitivity in obese insulin-resistant subjects and subjects with type 2 diabetes (1). Experimental elevation of plasma nonesterified fatty acid (NEFA) in humans using intravenous lipid infusion was associated with rapid deposition of IMTG associated with the development of insulin resistance within 4 h (2). Similarly, high-fat diet for 3 days in healthy volunteers induced IMTG accumulation associated with impaired insulin sensitivity (3). It has been demonstrated that subjects with type 2 diabetes have increased postprandial IMTG deposition (4), but the mechanisms leading to this phenomenon have not been elucidated. Impaired muscle fatty acid oxidation has been shown during fasting and during exercise in obese insulin-resistant subjects and subjects with type 2 diabetes (5–7). However, impaired muscle NEFA oxidation was associated with impaired NEFA uptake in the later studies, and suppression of muscle fatty acid oxidation by insulin was reduced in insulin-resistant individuals (8). During similar intravenous fat load, NEFA levels were higher in subjects with impaired glucose tolerance, a phenomenon that is significantly correlated with lipid-induced insulin resistance and impaired β-cell function in vivo (8). We demonstrated that this “impaired NEFA tolerance” during intravenous fat load is associated with increased whole-body palmitate oxidation in offspring of parents with type 2 diabetes (9). Recently, we showed that subjects with type 2 diabetes have increased postprandial NEFA appearance and whole-body oxidation rates without or with acute correction of hyperglycemia using intravenous insulin infusion to clamp glucose level (10).

The aim of the current study was to determine whether postprandial NEFA uptake in thigh skeletal muscles is increased in men with established but well-controlled type 2 diabetes compared with healthy men without a family history of type 2 diabetes. Our hypothesis was that increased postprandial NEFA appearance is associated with increased NEFA uptake in thigh skeletal muscle of men with type 2 diabetes. Our study design also allowed us, as a secondary objective, to determine postprandial leg subcutaneous adipose tissue NEFA uptake that may be a potential mechanism for increased postprandial plasma NEFA levels in type 2 diabetes.

RESEARCH DESIGN AND METHODS

Ethics statement. Informed written consent was obtained from all participants in accordance with the Declaration of Helsinki, and the protocol received approval from the Human Ethics Committee of the Centre Hospitalier Université de Sherbrooke.

Subjects. Seven healthy men without glucose intolerance (controls subjects [CON]), based on a 75-g oral glucose tolerance test, and seven men with established type 2 diabetes aged between 15 and 60 years participated in this metabolic study (Table 1). None of the participants had a current medical condition known to affect lipid levels or insulin sensitivity (other than type 2 diabetes), and participants had no known cardiovascular disease. Type 2 diabetic subjects were well controlled (AIC <7.5%) on diet alone and/or with only one oral hypoglycemic agent (sulfonylurea or metformin). Oral hypoglycemic and antihypertensive drugs, when taken, were stopped for 2 days before the study. Capillary blood glucose and blood pressure were monitored four times a day and daily, respectively, during this period. Statin therapy,
The fat drink was prepared by sonication of soybean oil (54 g/L), dried nonfat milk (263 g/L), egg phospholipids (0.18 g/L), and water (54 g/L). Tissue NEFA uptake was calculated by multiplying appropriate density (skeletal muscle: 1.0597 g/mL; adipose tissue: 0.9196 g/mL; Roche, Mississauga, Canada) to prevent in vitro TG hydrolysis (16).

Radioactivity assays. Glucose, insulin, total NEFA, and TGs were measured as described (9). Fractional plasma 18FTHA and metabolites were determined by thin-layer chromatography, as previously described (17).

PET/CT images analysis. All image-derived input functions and the full-width at half-maximum were drawn on both the left and right femoral arteries over three consecutive planes, with an interval of 4 cm between planes. Corrections were applied on input function for partial volume and tissue-to-artery spillover of radioactivity as described previously (18). The maximum value of the four highest adjacent pixels (from arterial uptake) of the tracer image was recorded. Regions of interest were drawn on the skeletal muscle (quadriceps femoris [QF] and biceps femoris [BF], e.g., predominantly white and red muscle fibers, respectively) and subcutaneous fat of the femoral region to generate tissue time-radioactivity curves (Supplementary Fig. 1B), as recently performed by others to determine adipose tissue NEFA uptake during fasting (19). The regions of interest were first defined from the transaxial CT slices and then copied to both the 14Cacetate and 18FTHA image sequences. Large vessels were avoided when outlining the muscle areas.

Calculations. Muscle blood flow index (the first-pass tissue fractional extraction, K1 in min−1, of 14Cacetate) and muscle oxidative metabolism index (the rapid fractional tissue clearance, k2 in min−1, of 14Cacetate) were estimated from 14Cacetate using a three-compartment model (13,20,21), as previously published by our group (18). At relatively low tissue blood flow rates as occurring in resting skeletal muscles, first-pass tissue fractional extraction of arterial 14Cacetate is close to 1 and provides a reliable index of tissue perfusion (22). Determination of tissue oxidative metabolism using the 14Cacetate method is based on the following assumptions (13): 1) acetate enters the Krebs cycle freely after rapid conversion into acetyl-CoA; 2) other acetate metabolic pathways (e.g., de novo lipogenesis, amino acid transcarboxylation) are relatively slow compared with the Krebs cycle carbon fluxes; 3) carbon flow from the Krebs cycle to acetyl-CoA is directly coupled with the production of reducing equivalents; 4) the Krebs cycle contribution to the production of reducing equivalents is stable and accounts for approximately two-thirds of total production; and 5) the production of reducing equivalents is tightly coupled with oxygen consumption.

The nonmetabolized fraction of 18FTHA was used to correct the plasma input function (Supplementary Fig. 1A) and plasma and femoral tissue time-radioactivity curves were analyzed graphically using the Patlak linearization method (Supplementary Fig. 1C) (17,18). The slope of the plot in the graphical analysis is equal to the tissue NEFA fractional extraction constant of 18FTHA (K1). K2 was corrected for tissue density by multiplying the constant by the appropriate density (skeletal muscle: 1.0597 g/mL; adipose tissue: 0.9196 g/mL). Tissue NEFA uptake was calculated by multiplying K1 by mean plasma NEFA concentration during 18FTHA PET imaging protocol, with a lumped constant of 1 when compared with endogenous plasma NEFA.

Statistical analysis. Data are expressed as mean ± SEM. Unpaired Student t test was used to compare differences between CON and type 2 diabetic subjects. Two-way ANOVA with group, time, and interaction as independent variables was used to analyze differences in plasma glucose, insulin, NEFA, and TGs throughout the protocol. Spearman correlation coefficients were used to determine correlation between variables. A two-tailed P value of <0.05 was considered significant. All analyses were performed with the SAS software for Windows, version 9.1.3 (SAS Institute, Cary, NC) or GraphPad Prism version 5.00 for Windows (San Diego, CA).

RESULTS

Participant characteristics and plasma metabolites and insulin levels. Type 2 diabetic subjects were older and tended to have higher body fat than CON. Type 2 diabetic subjects also had significantly higher fasting plasma glucose, insulin, total NEFA, and TGs.
glucose levels and tended to have increased fasting plasma insulin, NEFA, and LDL cholesterol levels.

Plasma glucose was higher in type 2 diabetic subjects than in CON throughout the metabolic study (Fig. 2A) (ANOVA $P < 0.01$) with significant group $\times$ time interaction due to the clamp procedure in the last 3 h that abolished difference between the two groups during the PET/CT acquisition period (ANOVA $P = 0.45$). Plasma insulin, NEFA, and TGs (Fig. 2B, C, and D, respectively) were also significantly higher in type 2 diabetic subjects than in CON (all ANOVA $P < 0.01$) without significant group $\times$ time interaction.

**Skeletal muscle blood flow and oxidative metabolic indexes.** QF (predominantly white fibers) and BF (predominantly red fibers) blood flow indexes (Fig. 3A and B) tended to be lower in type 2 diabetic subjects than in CON (QF: $0.21 \pm 0.07$ vs. $0.32 \pm 0.04$ min, $P = 0.17$; BF: $0.22 \pm 0.05$ vs. $0.33 \pm 0.05$ min, $P = 0.17$). There was no significant difference in blood flow index between QF and BF in both groups of participants. Skeletal muscle oxidative metabolism indexes (Fig. 3C and D) were similar in type 2 diabetic subjects and CON (QF: $0.27 \pm 0.11$ vs. $0.44 \pm 0.24$ min, $P = 0.93$; BF: $0.15 \pm 0.09$ vs. $0.20 \pm 0.08$ min, $P = 0.66$).

**Thigh skeletal muscle and subcutaneous adipose tissue NEFA fractional extraction and net NEFA uptake.** NEFA fractional extraction in thigh skeletal muscles (Fig. 4A and B) was significantly decreased (by $\sim$56%) in type 2 diabetic subjects versus CON (QF: $0.019 \pm 0.003$ vs. $0.040 \pm 0.008$ min, $P = 0.03$; BF: $0.019 \pm 0.003$ vs. $0.045 \pm 0.011$ min, $P = 0.04$) and tended to be decreased

![FIG. 1. Study protocol. ct, computed tomography acquisition; dyn, dynamic PET acquisition. The dots depict timing of blood samples.](image1)

![FIG. 2. Plasma glucose (A), insulin (B), NEFAs (C), and TGs (D) over time during the experimental protocol in the CON (○) and type 2 diabetic participants (●). Data are means ± SEM.](image2)
POSTPRANDIAL NEFA METABOLISM IN TYPE 2 DIABETES

by ~47% in subcutaneous fat in type 2 diabetic subjects versus CON (Fig. 4C: 0.010 ± 0.002 vs. 0.018 ± 0.004 min, P = 0.07). However, NEFA uptake in thigh skeletal muscles (Fig. 4D and E) was similar in type 2 diabetic subjects and CON (QF: 1.47 ± 0.23 vs. 1.37 ± 0.24 nmol · g⁻¹ · min⁻¹, P = 0.77; BF: 1.54 ± 0.26 vs. 1.46 ± 0.28 nmol · g⁻¹ · min⁻¹, P = 0.85). NEFA uptake in subcutaneous adipose tissue was also similar in type 2 diabetic subjects versus CON (Fig. 4F: 0.67 ± 0.15 vs. 0.52 ± 0.10 nmol · g⁻¹ · min⁻¹, P = 0.47). NEFA fractional extraction in leg subcutaneous fat was negatively correlated with BMI (Fig. 5A; r = −0.69, P < 0.01), but not with nonlean mass (r = −0.30, P = 0.30) or % fat mass (r = −0.34, P = 0.23). NEFA fractional extraction in skeletal muscles was positively correlated with muscle blood flow index (Fig. 5B and C; QF: r = 0.70, P = 0.02; BF: r = 0.71, P = 0.02). We found a significant correlation between muscle blood flow index and NEFA fractional extraction in healthy subjects but not in subjects with type 2 diabetes in both muscles (QF: r = 0.88, P = 0.02, and r = 0.33, P = 0.50, in CON and type 2 diabetic subjects, respectively; BF: r = 0.89, P < 0.001, and r = 0.27, P = 0.66, in CON and type 2 diabetic subjects, respectively). There was no significant difference in NEFA fractional extraction and NEFA uptake between QF and BF in both groups of participants. We found no significant relationship between muscle blood flow index and net muscle NEFA uptake (QF: r = −0.14, P = 0.71; BF: r = 0.54, P = 0.11) or between muscle NEFA fractional extraction and plasma NEFA level or muscle NEFA delivery (NEFA × muscle NEFA blood flow index) (not shown).

DISCUSSION
The current study demonstrated that postprandial NEFA uptake in femoral skeletal muscle and subcutaneous adipose tissue is similar in type 2 diabetic subjects compared with healthy subjects despite increased circulating NEFA levels in the former. The rate of total muscle NEFA uptake reported herein using 18FTHA PET/CT scanning in healthy men is compatible with the range of oxidative and nonoxidative muscle NEFA uptake reported during fasting by other groups using stable isotopic tracers and muscle biopsies or arterio-venous gradient (23,24). However, direct comparison between our technique and the later is difficult, since the current study reports total muscle NEFA uptake during the postprandial state. Remarkably, there was reduced muscle NEFA fractional extraction in type 2 diabetes that was directly related to reduction in muscle blood flow index during the postprandial state. This result was observed despite acute correction of postprandial hyperglycemia in type 2 diabetes using the insulin clamp. The present results suggest that the circulating NEFA pool is an unlikely source for the excess postprandial IMTG deposition observed in type 2 diabetes (4). Our results lend indirect support for an important role of excessive uptake of dietary TG in muscle in type 2 diabetes, as we recently demonstrated in the myocardium of diabetic rats (17).

Lipotoxicity refers to the process leading to end-organ damage and/or dysfunction after excess exposure to fatty acids and was first coined in the context of fat-induced insulin resistance and impaired glucose-stimulated insulin secretion leading to type 2 diabetes. Increased postprandial NEFA appearance rate is one of the mechanisms that may potentially lead to increased non-adipose tissue NEFA uptake and fatty acid exposure in type 2 diabetic subjects (25–29). In the current study, we found no increase in net skeletal muscle NEFA uptake despite the expected increase in circulating NEFA levels during the
postprandial state in type 2 diabetes, a phenomenon attributable to significantly reduced muscle NEFA fractional extraction. The results of the current study apply only to fully established type 2 diabetes, and we cannot exclude that muscle plasma NEFA uptake plays a role in muscle TG accretion and lipotoxicity earlier in the natural history of type 2 diabetes. Turpeinen et al. (15) showed, also using the \(^{18}\)FTHA PET method, that glucose-intolerant subjects display decreased skeletal muscle NEFA uptake during fasting. In the later study, this was attributable to significant reduction in muscle NEFA fractional extraction with similar NEFA levels in subjects with impaired glucose tolerance. Kelley and Simoneau (28), using arterio-venous balance across the leg, found significant reduction of \([3H]\) oleate fractional extraction associated with reduced fat oxidation, as assessed by limb indirect calorimetry, at fasting and after intake of a mixed meal in type 2 diabetes. Because circulating NEFA levels were higher, these authors found that net NEFA uptake was nevertheless higher across the leg in type 2 diabetes. These differences between type 2 diabetic subjects and control subjects in leg NEFA uptake and oxidation tended to be reduced toward the end of the 6-h postprandial period. Plasma glucose levels were very high (between 10 and 13 mmol/L) at fasting and during the postprandial period in type 2 diabetes in the latter study, associated with increased leg glucose oxidation that may lead to reciprocal reduction in fatty acid oxidation (30). Finally, it should be noted that results from limb arterio-venous balance studies may also be influenced by tissues other than skeletal muscles.

We found a direct relationship between blood flow index and skeletal muscle NEFA fractional extraction in the current study, a relationship that was more apparent in healthy individuals than in subjects with type 2 diabetes. Insulin-resistant states are associated with reduced post-prandial muscle blood flow (31), perhaps because of

**FIG. 4.** NEFA fractional extraction (A, B, and C) and net NEFA uptake (D, E, and F) in QF, BF, and subcutaneous fat, respectively, in the type 2 diabetic (T2D) participants (■) and CON (□). Data are means ± SEM. *P < 0.05 by unpaired Student t test.
impaired insulin-mediated capillary recruitment (32,33). It was shown in some (32,33), but not other studies (34,35), that muscle blood flow may be rate limiting for muscle glucose uptake, at least at higher levels of insulin sensitivity. We found no significant relationship between muscle blood flow index and net muscle NEFA uptake or between muscle NEFA fractional extraction and plasma NEFA level or muscle NEFA, demonstrating no apparent saturation of muscle NEFA transport capacity in the present experimental conditions. However, it is not possible to establish a causal relationship between reduced muscle blood flow index and reduction in muscle NEFA fractional extraction in subjects with type 2 diabetes, since other sources of fatty acids such as excess circulating TGs may compete for muscle intracellular NEFA transport, leading to apparent reduction of NEFA fractional extraction.

$^{18}$FTHA has been used previously to determine abdominal adipose tissue NEFA uptake in men during fasting (19). In contrast to the clear reduction of chylomicron-derived fatty acid uptake in subcutaneous adipose tissues of insulin-resistant and type 2 diabetic individuals (25–27,29), we found that postprandial adipose tissue uptake of plasma NEFA was virtually identical in type 2 diabetic subjects and CON. However, we found that type 2 diabetic participants tended to have lower subcutaneous adipose tissue NEFA fractional extraction than CON. However, adipose tissue NEFA fractional extraction was inversely associated with BMI in the current study, a phenomenon perhaps attributable to reduced postprandial adipose tissue blood flow with obesity. In obese and/or insulin-resistant individuals, fasting as well as postprandial increase in subcutaneous adipose tissue blood flow is lower than in healthy normal-weight subjects (36–41). Reduction of postprandial adipose tissue blood flow correlates with impaired insulin sensitivity throughout the natural history of type 2 diabetes (42). Impaired postprandial adipose tissue blood flow has been related, at least in part, to impaired adipose tissue uptake of chylomicron-derived fatty acids in morbid obesity (43). Unfortunately, we could not assess adipose tissue blood flow index in the current study given the very low uptake of $^{[11C]}$acetate in this tissue. We could not find significant reduction of muscle oxidative metabolism in type 2 diabetes. This result contrasts with the results of others who found reduced skeletal muscle oxidative phosphorylation in prediabetic insulin-resistant individuals (44–46). The presence of impaired skeletal muscle mitochondrial oxidative capacity in type 2 diabetes is controversial (47,48). In subjects with type 2 diabetes, whole-body postprandial NEFA oxidation is increased, not reduced, whether glucose level is acutely corrected or not with insulin clamp (10). It should be noted that the $^{[11C]}$acetate method we used is based on measurement of $^{11}$CO$_2$ production from the $^{[11C]}$acetyl-CoA intracellular pool (e.g., it is a measure of the Krebs cycle) (13). Mitochondrial uncoupling in vivo in skeletal muscle in type 2 diabetes could perhaps explain normal or high Krebs cycle carbon flux with reduced ATP production. Because our sample size was small, we cannot exclude that the lack of difference between type 2 diabetic subjects and CON in muscle oxidative metabolism in the current study could be due to a type 2 error.

The steady-state design of our postprandial study did not fully reproduce physiological meal intake and may lead to lower late postprandial overshoot of plasma NEFA level (49). This may have attenuated differences in skeletal muscle NEFA uptake between type 2 diabetes and CON in the current study. On the other hand, acute correction of postprandial hyperglycemia in type 2 diabetes is expected to increase fatty acid oxidation at the expense of glucose oxidation (11), leading to increased muscle fatty acid use in the latter. We used an identical postprandial protocol in a recent study without and with acute correction of hyperglycemia with insulin infusion and found nevertheless that type 2 diabetic subjects had higher whole-body postprandial plasma NEFA oxidation than CON in both conditions. Another limitation of our study is that $^{18}$FTHA, a reliable tracer of net tissue NEFA uptake (14,15,50),
cannot distinguish oxidative versus nonoxidative tissue NEFA metabolism. We previously found elevated whole-body NEFA and net fatty acid oxidation during the postprandial state in type 2 diabetes (10). It is therefore possible that increased postprandial plasma NEFA oxidation in type 2 diabetes is the result of increased uptake and oxidation in other lean organs such as the heart and the liver.

In conclusion, similar postprandial skeletal muscle NEFA uptake in type 2 diabetic and control subjects in this study provides evidence against a major role for increased plasma NEFA in the postprandial fat accretion observed in muscles in type 2 diabetes. Impaired skeletal muscle blood flow leading to impaired muscle NEFA fractional extraction may explain this finding. The results of the current study suggest that other sources of excess fatty acid uptake such as circulating lipoprotein-TG may be responsible for acute postprandial IMMCG accumulation in type 2 diabetes.

ACKNOWLEDGMENTS
This work was supported by a grant from the Canadian Institutes of Health Research (CIHR-MOP 530904) and was performed at the Centre de recherche clinique Étienne-Le Bel, a research center funded by the Fonds de la recherche en santé du Québec (FRSQ). A.C.C. is currently the recipient of a Fonds de recherche en santé du Québec Senior Scholarship Award. S.M.L. is the recipient of a Canadian Diabetes Association Doctoral Studentship Award.

No potential conflicts of interest relevant to this article were reported.

S.M.L. researched data and wrote the manuscript. E.C., T.G.-L., F.F., R.O., and R.L. researched data. B.G. and E.E.T. contributed to discussion. A.C.C. wrote the manuscript.

REFERENCES