Circulating Palmitoleate Strongly and Independently Predicts Insulin Sensitivity in Humans

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OBJECTIVE — We investigated whether palmitoleate, which prevents insulin resistance in mice, predicts insulin sensitivity in humans.

RESEARCH DESIGN AND METHODS — The fasting fatty acid pattern in the plasma free fatty acid (FFA) fraction was determined in 100 subjects at increased risk for type 2 diabetes. Insulin sensitivity was estimated during an oral glucose tolerance test (OGTT) at baseline and after 9 months of lifestyle intervention and measured during the euglycemic-hyperinsulinemic clamp (n = 79).

RESULTS — Circulating palmitoleate (OGTT:F ratio = 8.2, P = 0.005; clamp:F ratio = 7.8, P = 0.007) but not total FFAs (OGTT:F ratio = 0.6, P = 0.42; clamp:F ratio = 0.7, P = 0.40) correlated positively with insulin sensitivity, independently of age, sex, and adiposity. High baseline palmitoleate predicted a larger increase in insulin sensitivity. For 1-SD increase in palmitoleate, the odds ratio for being in the highest versus the lowest tertile of adjusted change in insulin sensitivity was 2.35 (95% CI 1.16–5.35).

CONCLUSIONS — Circulating palmitoleate strongly and independently predicts insulin sensitivity, suggesting that it plays an important role in the pathophysiology of insulin resistance in humans.

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Free fatty acids (FFAs) are considered to link obesity with insulin resistance and type 2 diabetes (1,2). Mechanisms include intracellular accumulation of lipotoxic metabolites, such as long-chain fatty acyl-CoA, ceramides, and diacylglycerol, which interfere with insulin signaling (2) and signaling via membrane toll-like receptor 4 (3). However, a significant relationship between total FFA levels and insulin resistance is not found in all studies (4–6).

Most recently, the fatty acid palmitoleate (C16:1n7) was found to increase insulin action in skeletal muscle and to prevent hepatosteatosis in mice, thus representing a link between adipose tissue and systemic metabolism (7). In the present study, we investigated whether palmitoleate may also be a determinant of insulin sensitivity in humans.

RESEARCH DESIGN AND METHODS

— Data from 100 Caucasians of the Tübingen Lifestyle Intervention Program (8) were analyzed. Subjects underwent measurements at baseline and after 9 months of lifestyle intervention (≥3 h of moderate sports per week, intake of calories from fat <30%, intake of fibers ≥15 g/1,000 kcal, intake of saturated fat ≤10%). Informed written consent was obtained from all participants, and an ethics committee approved the protocol. Body fat was measured by bioelectrical impedance, total and visceral fat by magnetic resonance tomography, and liver fat by ^{1}H magnetic resonance spectroscopy (8). Insulin sensitivity was estimated from a 75-g oral glucose tolerance test (OGTT), as proposed by Matsuda and DeFronzo [$10{,}000/\sqrt{(Ins_{mean} \cdot Gluc_{mean} \cdot Ins_0 \cdot Gluc_0)}$] and additionally measured by the euglycemic clamp (8). The fatty acids in the plasma FFA fraction were measured as previously described (supplemental Table 1 in the online appendix at http://care.diabetesjournals.org/cgi/content/full/dc09-0544/DC1) (9). Forward stepwise multivariate linear and logistic regressions were performed.

RESULTS

— Subject characteristics are shown in supplemental Table 2. At baseline, insulin sensitivity correlated inversely with body weight, BMI, waist circumference, total body fat, visceral fat, and liver fat (all $P < 0.0001$). Total fasting FFA levels were lower in males (P = 0.0002). They were not associated with age or with measures of adiposity, liver fat (all adjusted $P > 0.09$), or insulin sensitivity (OGTT:F ratio = 0.6, P = 0.42; clamp:F ratio = 0.7; P = 0.40) independently of sex, age, and body fat.

Circulating palmitoleate was lower in males (4.38 ± 0.19 vs. 5.03 ± 0.14%, P = 0.007) and not associated with age, or with measures of adiposity such as body weight, BMI, waist circumference, or total and visceral fat, adjusted for sex and age (all $P > 0.23$). Because palmitoleate in serum cholesteryl esters correlated with high-sensitivity C-reactive protein (hsCRP) levels (10), we also investigated the relationship between circulating palmitoleate and adjusted hsCRP levels. No significant relationship was found (P = 0.44). A weak negative correlation between palmitoleate and liver fat was observed after adjustment for age, sex, and body fat (F ratio = 3.7; P = 0.057). In contrast, palmitoleate correlated positively with insulin sensitivity

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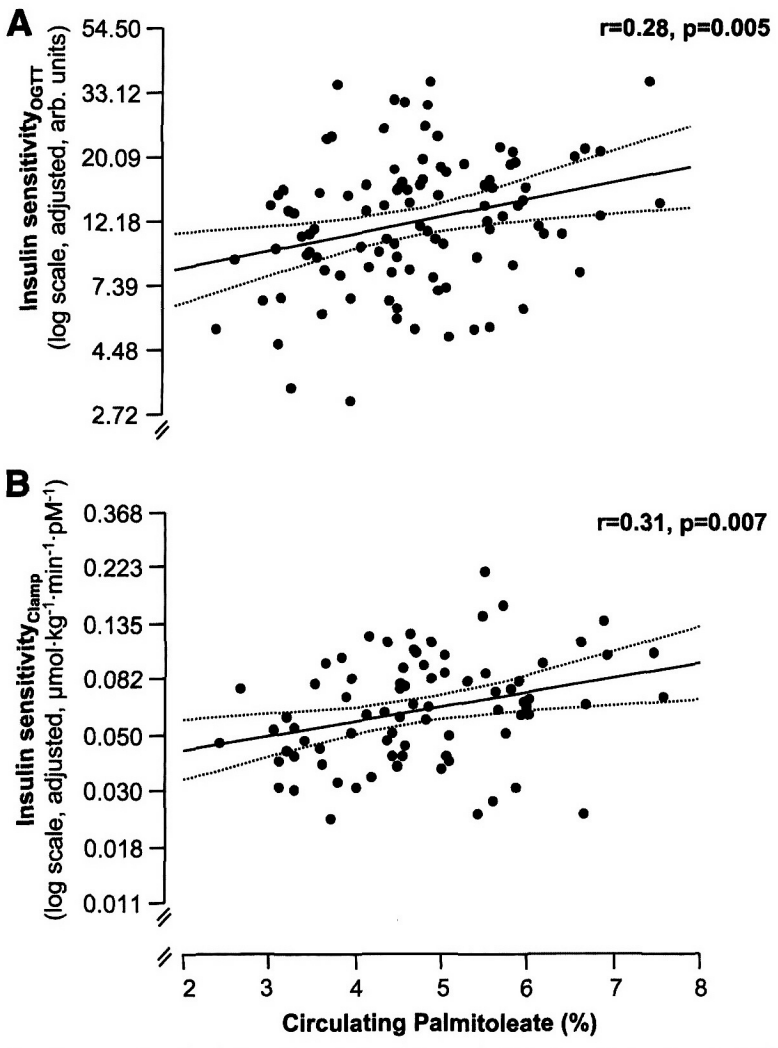

Figure 1—*Cross-sectional relationships of circulating palmitoleate with insulin sensitivity estimated from the OGTT* (A) *and measured by the clamp* (B) *at baseline. Insulin sensitivity was adjusted for age, sex, and body fat in multivariate linear regression models (regression line and 95% CI). arb. units, arbitrary units.*

(OGTT:*F* Ratio = 8.2, Fig. 1*A*; clamp:*F* ratio = 7.8, Fig. 1*B*) independently of sex, age, and body fat. Additional adjustment for visceral fat only moderately affected these relationships (P = 0.017 and P = 0.019).

During the 9 months of lifestyle intervention, insulin sensitivity and circulating palmitoleate increased ($P < 0.0001$, supplemental Tables 1 and 2). Change in palmitoleate did not correlate with changes in insulin sensitivity in all subjects (n = 95, OGTT P = 0.67, and n = 38, clamp P = 0.56). However, a positive relationship was observed in subjects in the upper tertile of insulin sensitivity (OGTT) at baseline (r = 0.41, P = 0.03), suggesting that in subjects starting with low insulin sensitivity, the multiple benefits of lifestyle interaction such as reduction in adiposity or increased exercise intensity have a stronger impact on insulin sensitivity than change in palmitoleate levels. In agreement with the analyses at baseline, at the follow-up visit, palmitoleate levels also correlated positively with adjusted insulin sensitivity (n = 95, OGTT:*F* ratio = 5.0; P = 0.029).

In forward stepwise linear regression analyses including palmitoleate at baseline, age, sex, insulin sensitivity at baseline, and body fat at baseline and at follow-up, high palmitoleate levels predicted a larger increase in insulin sensitivity (P = 0.02, supplemental Table 3). After dividing subjects into tertiles by the observed change in insulin sensitivity, for 1-SD increase in circulating palmitoleate at baseline, the odds ratio of subjects for being in the highest versus the lowest tertile of change in insulin sensitivity was 2.35 (95% CI 1.16–5.35).

CONCLUSIONS— We found that circulating palmitoleate was a determinant of insulin sensitivity both estimated from the OGTT and measured by the clamp. Furthermore, in subjects with high palmitoleate at baseline, there was a higher chance to observe an increase in insulin sensitivity, independently of the change in adiposity, compared with subjects with low levels. These novel data strongly support that palmitoleate may also be involved in the regulation of insulin sensitivity in humans.

In animals, palmitoleate infusion decreased expression of hepatic lipogenic enzymes, thus possibly regulating liver fat (7), an important determinant of insulin sensitivity in humans (11,12). We observed a weak negative relationship between circulating palmitoleate and liver fat, suggesting that, in humans, the effects of palmitoleate on the regulation of insulin sensitivity may be more pronounced than on hepatic steatosis.

What mechanisms are involved in the determination of circulating palmitoleate? The lipid chaperons fatty acid–binding protein (FABP)-4 and -5 were found to suppress the biosynthesis of palmitoleate (7). FABPs induce lipolysis in adipocytes and inflammatory pathways in macrophages, particularly in visceral obesity (13). However, FABP expression was not elevated in visceral obesity (14) and, in the present study, palmitoleate levels did not correlate with visceral fat mass, suggesting that increase in this fat compartment does not appreciably determine palmitoleate levels. Another regulator of palmitoleate may be diet. However, dietary levels of palmitoleate are very low. Therefore, palmitoleate levels are most probably not largely affected by the diet, but mainly by de novo lipogenesis in adipose tissue (7). In addition, stearoyl-CoA desaturase-1, the key enzyme in the biosynthesis of palmitoleate from palmitate, may determine palmitoleate levels. This is supported by data showing that thiazolidinedione treatment increased insulin sensitivity, circulating palmitoleate, and expression and activity of stearoyl-CoA desaturase-1 in adipose tissue (15).

In conclusion, particularly the cross-sectional analyses with measurements of insulin sensitivity from both the OGTT and the clamp suggest that palmitoleate strongly and independently of adiposity determines insulin sensitivity. Therefore, circulating palmitoleate may also play an important role in the pathophysiology of insulin resistance in humans.

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