Differential Effects of *Cis* and *Trans* Fatty Acids on Insulin Release From Isolated Mouse Islets

Kirsten Kudahl Alstrup, Søren Gregersen, Heidi Majgaard Jensen, Janus Laust Thomsen, and Kjeld Hermansen

In vitro and in vivo studies in animals have shown that elevated levels of free fatty acids (FFAs) induce impaired β-cell function corresponding to the abnormalities observed in non–insulin-dependent diabetes mellitus (NIDDM). Previously, it was demonstrated that the chain length and degree of unsaturation are of importance for the insulinotropic effect of fatty acids. However, it is not known if the spatial configuration of the fatty acid influences β-cell function. The present study examines whether *cis* and *trans* fatty acids acutely influence insulin release and glucose oxidation in isolated mouse islets in the same way and to the same extent. Thus, we studied the impact of both *cis* and *trans* forms of C 18:1 fatty acids. We found that *cis* and *trans* vaccenic acid (*cis* and *trans* C 18:1 Δ11), as well as oleic acid (*cis* C 18:1 Δ9) and elaidic acid (*trans* 18:1 Δ9), caused a dose-dependent increase in glucose (16.7 mmol/L)-stimulated insulin secretion during static islet incubations. The maximal stimulatory effect for *cis* and *trans* vaccenic acid and for oleic and elaidic acid was observed at concentrations of 2.0 and 3.0 mmol/L, respectively. The *trans* isomers, *trans* vaccenic and elaidic acid, elicited a higher maximal insulin output than the respective *cis* isomers, *cis* vaccenic and oleic acid. In the presence of another insulin secretagogue, L-leucine, *trans* vaccenic but not elaidic acid caused a higher response than their *cis* isomeric fatty acids. The higher potency of *trans* fatty acids compared with the *cis* forms was confirmed in perifusion experiments. Both *cis* and *trans* C 18:1 fatty acids stimulated insulin secretion in a glucose-dependent manner. Also, glucose oxidation was influenced differentially by the isomers of fatty acids. Glucose oxidation at 16.7 mmol/L glucose was significantly inhibited by oleic and *cis* vaccenic acid compared with elaidic and *trans* vaccenic acid, respectively. In summary, our results demonstrate that the fatty acid spatial configuration modulates glucose oxidation and insulin secretion in mouse β cells.

NON–INSULIN-DEPENDENT diabetes mellitus (NIDDM) is characterized by insulin resistance and impaired β-cell function. NIDDM subjects are often obese and have elevated levels of free fatty acids (FFAs) in plasma.[1,2] The question arises as to whether FFAs may play a role in the pathogenesis of NIDDM, and consequently, a number of studies have been performed to clarify the influence of fatty acids on the endocrine pancreas.[3-8] It has been found that fatty acids acutely stimulate insulin secretion both in isolated rat islets[9,10] and in vivo in humans.[11] In contrast, long-term exposure of human and rodent islets to fatty acids in vitro[3-6,8] and in vivo[3,5,12] causes impaired insulin release in response to a high glucose concentration, whereas insulin hypersecretion occurs at low glucose concentrations.[4,5] These findings mimic the abnormalities found in NIDDM, where patients often present with hyperinsulinemia and hyperglycemia.

Interestingly, it appears that the chemical structure of fatty acids is of importance for the amount of insulin released. The acute insulinotropic effect increases with increasing chain length[13-15] and decreases with a higher degree of unsaturation.[15] Furthermore, the spatial configuration of fatty acids may also be of importance. Stein et al[15] have found that palmitelaidic acid (*trans* C 16:1 Δ9) tended to be more insulinotropic than the *cis* isomer in the perfused rat pancreas. In humans, a diet enriched in saturated or *trans* fatty acids seems to elevate postprandial insulin and C-peptide responses after a test meal. Since blood glucose was unaltered, the findings might be explained by increased insulin resistance; however, a differential effect of the isomeric fatty acids on the endocrine pancreas cannot be excluded.[16]

Trans fatty acids are unsaturated fatty acids differing from *cis* isomers only in the spatial configuration. In *trans* isomers, the carbon moieties on the two sides of a double bond provide a straight, closely packed configuration, whereas *cis* isomers have a bent configuration. Most natural fats and oils contain only *cis* isomers, but *trans* fatty acids are formed when liquid vegetable oils are partially hydrogenated to form margarine and shortenings, with elaidic acid (C 18:1 Δ9) as a major component. Furthermore, *trans* fatty acids are found in milk and in the body fat of ruminants, with *trans* vaccenic acid (C 18:1 Δ11) being the most predominant.[17,18]

The present investigation was performed to examine the acute effects on insulin release of the most common *trans* fatty acids (elaidic and *trans* vaccenic acid) and their corresponding *cis* isomers. Concomitantly, the influence of *cis* and *trans* fatty acids on islet glucose oxidation was studied.

MATERIALS AND METHODS

Isolation of Islets

Female Naval Medical Research Institute (NMRI) mice (Bomholtgaard Breeding and Research Center, Ry, Denmark) weighing 20 to 25 g were used. They were fed a standard pellet diet with tap water ad libitum before the experiments, and were maintained on a 12-hour dark/light cycle. The mice were handled in accordance with Danish law, with the approval of the Animal Experiments Inspectorate under the Ministry of Justice.

Islets were obtained using the collagenase digestion technique.[19,20] They were handpicked under a stereomicroscope and transferred for

From the Department of Endocrinology and Metabolism, Aarhus University Hospital, Aarhus, Denmark.

Submitted July 17, 1997; accepted July 17, 1998.

Supported by grants from the Aarhus University Research Foundation, Danish Medical Research Council, Institute of Experimental Clinical Research, Aarhus University, Faculty of Medicine, Aarhus University, Danish Diabetes Association, Novo Nordisk Foundation, Aarhus Amtssygehus Research Foundation, Mogens and Jenny Vissings Foundation, Velux Foundation, and A.P. Moeller and Chastine McKinney Moellers Foundation.

Address reprint requests to Kirsten Kudahl Alstrup, MD, Department of Endocrinology and Metabolism, Aarhus University Hospital, Tage-Hansens Gade 2, 8000 Aarhus C, Denmark.

0026-0495/99/4801-0005$03.00/0

overnight culture in RPMI 1640 supplemented with 10% (vol/vol) fetal calf serum, glutamine (2.06 mmol/L), penicillin G (100 IU/mL), and streptomycin (100 μg/mL) (all Gibco BRL, Paisley, UK) in an atmosphere of 95% humidified air/5% CO_2. The incubation experiments started with a 60-minute preincubation of the islets at 3.3 mmol/L in HEPES-buffered medium containing 125 mmol/L NaCl, 5.9 mmol/L KCl, 1.2 mmol/L $MgCl_2$, 1.28 mmol/L $CaCl_2$, 5.0 mmol/L $NaHCO_3$, 25 mmol/L HEPES (pH 7.4), and 0.1% bovine serum albumin ([BSA] fatty acid–free; Boehringer, Mannheim, Germany).

Incubation Studies

Single islets were incubated in 200 μL HEPES-buffered medium supplemented with 3.0% BSA, glucose (3.3 to 22.0 mmol/L), and fatty acids (*cis/trans* vaccenic acid, oleic acid, and elaidic acid; all Sigma Chemical, St Louis, MO). In some studies, L-leucine (5.0 mmol/L) was included in the medium. The islets used in each incubation experiment were obtained from six to nine animals. A stock solution of each fatty acid was prepared by mixing and heating to 80° to 90°C equimolar amounts of NaOH and fatty acids supplemented with distilled H_2O to a final concentration of 100 mmol/L. The stock solution was stored at −20°C. On the day of the experiment, a suitable amount of hot (80° to 90°C) stock solution was slowly added to a 37°C HEPES-buffered medium containing 3% BSA under magnetic stirring.

After a 60-minute incubation of the islets at 37°C, 100 μL incubation medium was removed for insulin analysis.

Perifusion Studies

After overnight culture, the islets were rinsed three times in HEPES-buffered medium containing 3.3 mmol/L glucose and 0.1% BSA. Perifusion experiments were performed in a four-chamber perifusion system or a six-chamber Brandel 2500 superfusion system (Brandel, Gaithersburg, MD). The islets were placed in BioGel P-4 (Bio-Rad, Richmond, CA) between two filters. The flow rate was 100 μL/min and the temperature 37°C. The islets were perifused in HEPES-buffered medium containing 3% BSA, FFAs, and glucose according to the protocols. At the end of the perifusion, carbacholine (Sigma Chemical) was added as a control. In the perifusion experiments, fractions were collected every 2 minutes and used for analysis of insulin.

Glucose Oxidation

Islet glucose oxidation was determined by measuring the formation of $^{14}CO_2$ from [U-^{14}C]glucose (Amersham Life Science, Amersham, UK).[21] Twenty islets were transferred in a volume of 30 μL HEPES-buffered medium (0.1% BSA and 3.3 mmol/L glucose) to 15-mL tubes, placed in a waterbath at 37°C, and preincubated for 45 minutes. To obtain the desired final concentrations of glucose, BSA, and fatty acids, 10 μL HEPES-buffered medium containing a surplus of BSA, glucose, and fatty acids together with 0.8 μCi [U-^{14}C]glucose was added. Another tube containing a Whatman GF/C filter (Whatman International, Maidstone, England) was immediately connected to the first tube, creating an airtight chamber, and the samples were incubated for 2 hours at 37°C in a shaking waterbath. One hundred microliters of 50% (wt/vol) phenethylamine (Merck, Darmstadt, Germany) in methanol was injected through the connecting tube to the filter and 100 μL 10% trichloroacetic acid was injected to the islets, stopping the metabolic reaction. Following the injection, the tube was immediately pushed together to close the injection hole. The tubes were left for 45 minutes at room temperature to allow the $^{14}CO_2$ produced to absorb to the filters containing phenethylamine. Blank incubations without islets were treated similarly. Radioactivity was determined by a liquid scintillation counter. The recovery of $^{14}CO_2$ as assessed with $NaH^{14}CO_3$ (Amersham Life Science) was 78% ± 3% (n = 12). Data are expressed as picomoles of oxidized glucose per 20 islets during 2 hours of incubation.

Insulin Assay

Insulin was determined by radioimmunoassay with the guinea pig antiporcine insulin antibody PNILGP4 (Novo Nordisk, Bagsvaerd, Denmark) and mono-^{125}I-(Tyr A14)–labeled human insulin (Novo Nordisk) as a tracer and rat insulin (Novo Nordisk) as a standard. Free and bound radioactivity were separated using ethanol. Fatty acids at the concentrations studied did not interfere with the assay. Interassay and intraassay variations were between 5% and 10%.

Statistical Analysis

For statistical comparisons in static experiments, ANOVA was used followed by the Newman-Keuls test. Data from perifusion experiments were analyzed first by ANOVA, and second by calculating the area under the curve (AUC) followed by a comparison using Student's unpaired two-tailed *t* test. Results are expressed as the mean ± SEM.

RESULTS

Effects of Cis *and* Trans *Vaccenic Acid on Insulin Secretion*

When islets were exposed to increasing concentrations (0.1 to 2.0 mmol/L) of *cis* or *trans* vaccenic acid in the presence of 16.7 mmol/L glucose, a concentration-dependent increase in insulin secretion was found (Fig 1). One to 3 mmol/L *cis* and *trans* vaccenic acid caused a significant increase in insulin release in the presence of 16.7 mmol/L glucose ($P < .05$, respectively). For both fatty acids, a maximal stimulatory effect was found at 2.0 mmol/L. A further increase in the fatty acids to 3.0 to 4.0 mmol/L caused a decrease in insulin release compared with 2.0 mmol/L. *Trans* vaccenic acid (1.0 to 3.0 mmol/L) potentiated insulin secretion to a larger extent than the equimolar concentration of *cis* vaccenic acid ($P < .05$). When islets were exposed to *cis* or *trans* vaccenic acid (0.1 to 4.0 mmol/L) at low glucose (3.3 mmol/L) for 1 hour, insulin secretion was unaffected (data not shown).

The greater potency on a molar basis of *trans* compared with *cis* vaccenic acid was confirmed in dynamic perifusion experiments performed at 16.7 mmol/L glucose. For perifusion, a submaximal stimulatory concentration of 1.0 mmol/L was chosen (Fig 2A and B). A monophasic insulin response to both *cis* and *trans* vaccenic acid appeared. A slow decline toward the prestimulatory level was found after omitting the fatty acids. With the addition of *cis* and *trans* vaccenic acid, the AUC differed significantly. A 37% larger AUC was observed in response to *trans* versus *cis* vaccenic acid (2,968 ± 146 *v* 2,167 ± 104 μU/mL · 30 min, $P < .003$). Carbacholine (0.1 mmol/L) subsequently elicited a similar insulin response irrespective of whether the islets were exposed to *cis* or *trans* vaccenic acid. This indicates that the islets were functionally intact at the end of the experiment.

Effects of Oleic Acid and Elaidic Acid on Insulin Secretion

Elaidic acid elicited a concentration-dependent increase in glucose (16.7 mmol/L)-stimulated insulin secretion when added in a concentration range between 0.5 and 3.0 mmol/L (Fig 3). For oleic acid, the increase in insulin secretion was only significant for 2.0 to 4.0 mmol/L fatty acid ($P < .05$). Maximal responses were obtained at a concentration of 3.0 mmol/L fatty

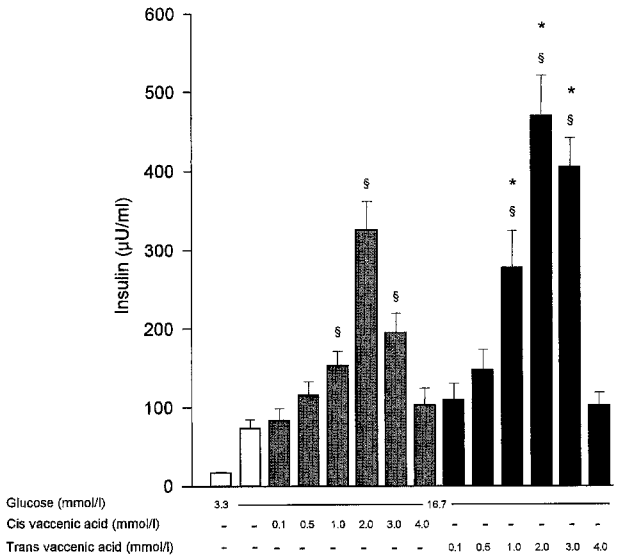

Fig 1. Effect of *cis* and *trans* vaccenic acid (0.1-4.0 mmol/L, respectively) on glucose-stimulated (16.7 mmol/L) insulin release from single mouse islets incubated for 60 minutes. Each bar represents the mean ± SEM of 40-46 islets. §$P < .05$ *v* control (without fatty acid). *$P < .05$ *v* equimolar level of the isomeric fatty acid (*cis* vaccenic acid).

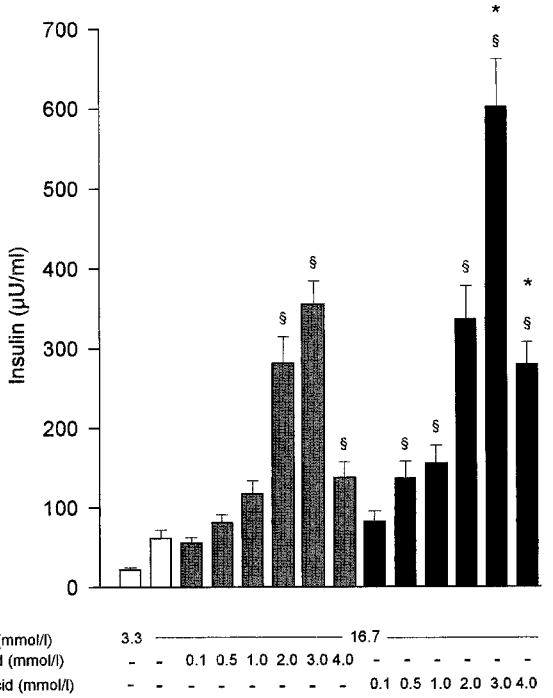

Fig 3. Effect of oleic and elaidic acid (0.1-4.0 mmol/L, respectively) on glucose-stimulated (16.7 mmol/L) insulin release from single mouse islets incubated for 60 minutes. Each bar represents the mean ± SEM of 40-45 islets. §$P < .05$ *v* control (without fatty acid). *$P < .05$ *v* equimolar level of the isomeric fatty acid (oleic acid).

acids in the presence of 16.7 mmol/L glucose. Again, we found that the *trans* isomer (elaidic acid) stimulated insulin secretion more potently than the *cis* isomer (oleic acid). The insulin response to 3.0 and 4.0 mmol/L elaidic acid was about twofold higher versus the equimolar concentration of oleic acid. ($P < .05$).

We also tested these fatty acids in perifusion experiments (Fig 4). The insulin response to oleic and elaidic acid (40 to 70 minutes) did not differ significantly (8,106 ± 297 *v* 10,114 ± 952 µU/mL, $P < .09$), but when the AUC was split into two (40 to 56 and 56 to 70 minutes), the AUC (56-70 minutes) for elaidic acid was significantly larger than the AUC (56 to 70 minutes)

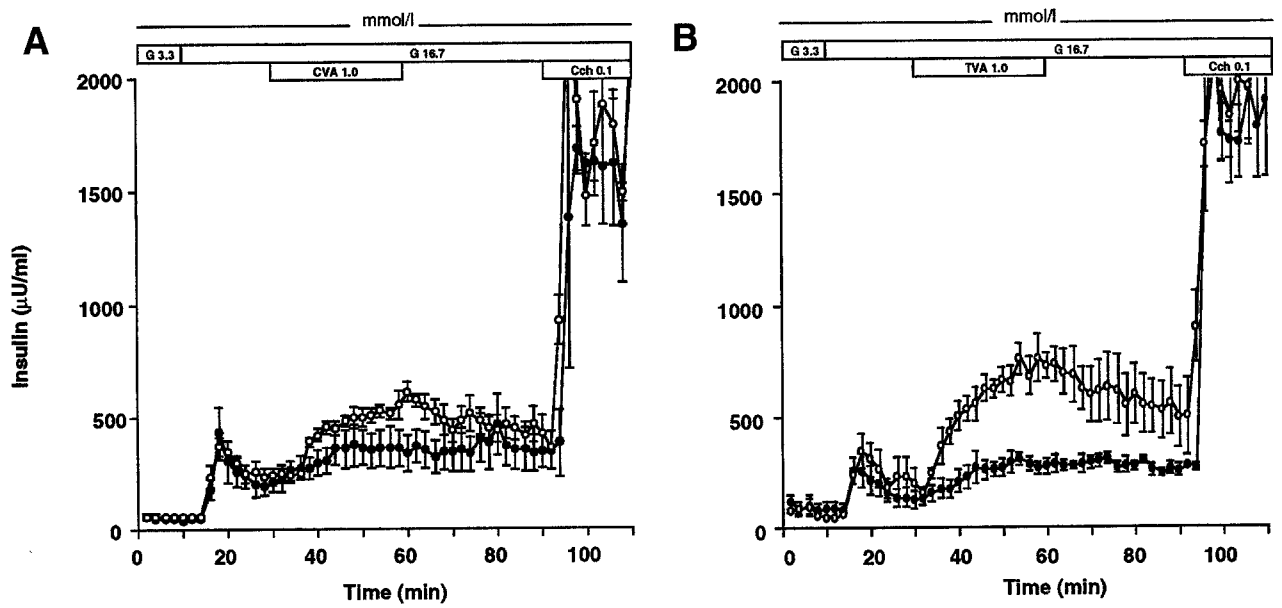

Fig 2. Effect of 1.0 mmol/L (A) *cis* vaccenic acid (CVA) or (B) *trans* vaccenic acid (TVA) on glucose (G)-stimulated (16.7 mmol/L) insulin secretion from perifused mouse islets. From 30 to 60 minutes, *cis* or *trans* vaccenic acid were added to the perifusion medium. The last 20 minutes, carbacholine (Cch) (0.1 mmol/L) was added. Results are the mean ± SEM (n = 4 perifusion chambers in each group, with each chamber containing 40 islets). ●, Control; ○, *trans* vaccenic acid.

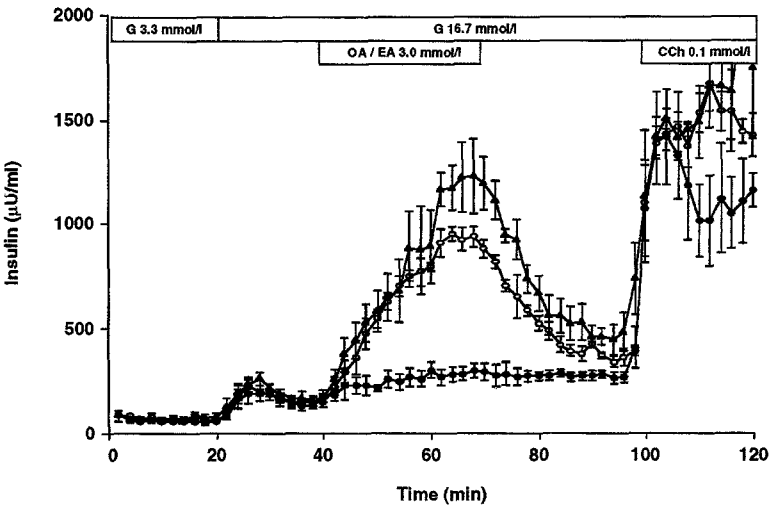

Fig 4. Effect of oleic and elaidic acid (3.0 mmol/L) on glucose (G)-stimulated (16.7 mmol/L) insulin secretion from perifused mouse islets. From 40 to 70 minutes, oleic or elaidic acid were added to the perifusion medium. The last 20 minutes, carbacholine (CCh) (0.1 mmol/L) was added. Results are the mean ± SEM (n = 4 perifusion chambers in each group, with each chamber containing 30 islets). ●, Control; ○, oleic acid; ▲, elaidic acid.

for oleic acid (6,731 ± 417 *v* 5,121 ± 193 µU/mL, $P < .05$), indicating that elaidic acid stimulates insulin secretion more potently than oleic acid after 15 minutes.

Effects of Glucose on Fatty Acid–Stimulated Insulin Secretion

The glucose dependency of the insulinotropic action of the fatty acids was subsequently studied (Fig 5a and b). As expected, glucose showed concentration-dependent stimulation of insulin secretion at and above 16.7 mmol/L ($P < .05$). We compared the molar potency of different fatty acids (3.0 mmol/L) at varying glucose concentrations. We found that *trans* fatty acids potentiated glucose-stimulated insulin secretion at and above 11.0 mmol/L glucose, whereas *cis* isomeric fatty acids only enhanced insulin release at 11.0 and 16.7 mmol/L glucose ($P < .05$, respectively). Again, *trans* fatty acids were more potent than the *cis* isomers: *trans* vaccenic acid (3.0 mmol/L) stimulated insulin release more potently than an equimolar concentration of *cis* vaccenic acid at glucose concentrations of 11.0 to 22.0 mmol/L ($P < .05$). Elaidic acid (3.0 mmol/L) elicited greater insulin release than an equimolar concentration of the corresponding *cis* isomers (oleic acid) at 11.0 and 16.7 mmol/L glucose ($P < .05$) (Fig 5B).

Effects of Cis *and* Trans *Fatty Acids on* L*-Leucine–Stimulated Insulin Release*

The effect of isomeric fatty acids on insulin release was also compared during 60-minute incubations in the presence of L-leucine (Table 1). It was found that 5.0 mmol/L L-leucine in combination with a nonstimulatory glucose concentration (4.0 mmol/L) enhanced insulin release ($P < .05$). When 3.0 mmol/L

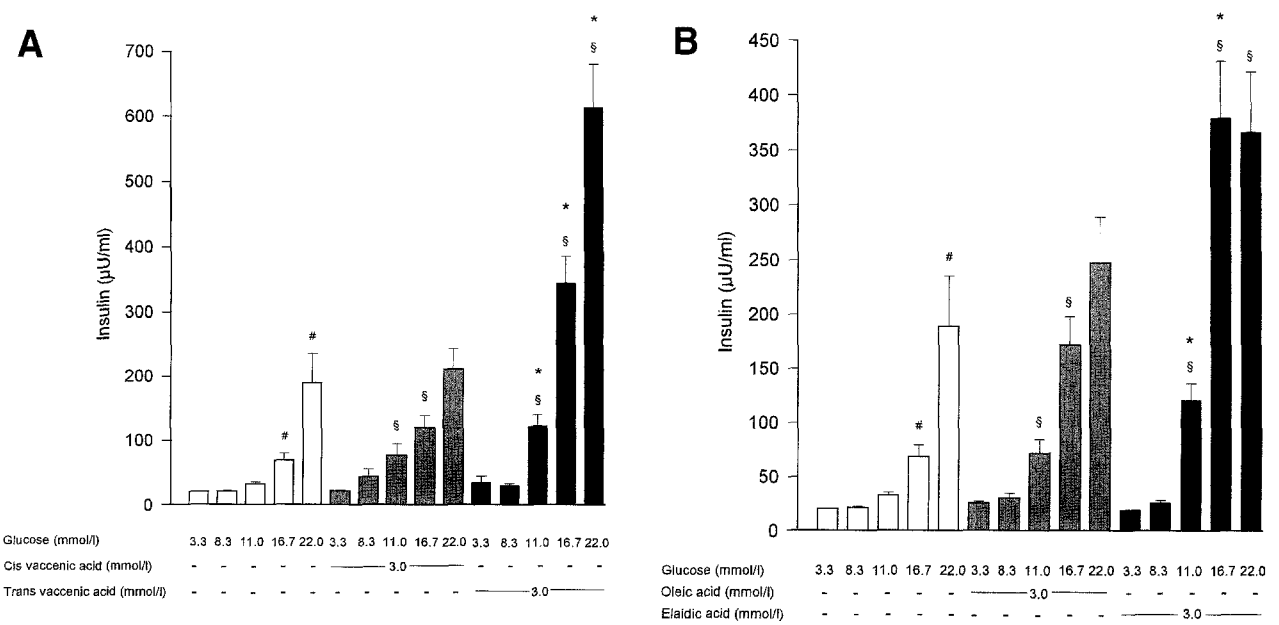

Fig 5. Effect of glucose (3.3-22.0 mmol/L) in the absence and presence of (A) 3.0 mmol/L *cis* or *trans* vaccenic acid or (B) 3.0 mmol/L oleic or elaidic acid. Single mouse islets were incubated for 60 minutes. Each bar represents the mean ± SEM of 20-24 islets. #$P < .05$ *v* low glucose (3.3 mmol/L) without added fatty acids. §$P < .05$ *v* control at the same glucose level without fatty acids. *$P < .05$ *v* equimolar level of the isomeric fatty acid at similar glucose concentrations.

isomeric fatty acid was added, a further increase in insulin release was observed. Again, we found that 3.0 mmol/L *trans* vaccenic acid potentiated insulin secretion significantly compared with 3.0 mmol/L *cis* vaccenic acid ($P < .05$). However, similar insulinotropic effects were found for oleic acid and elaidic acid in the presence of L-leucine (5 mmol/L).

Effects of Cis *and* Trans *Fatty Acids on Glucose Oxidation*

We did not find any influence of 3.0 mmol/L *trans* vaccenic acid and elaidic acid on glucose oxidation either at 3.3 or 16.7 mmol/L glucose (Fig 6). In contrast, the isomeric fatty acids (3.0 mmol/L *cis* vaccenic acid or oleic acid) caused a clear-cut suppression of glucose oxidation with 16.7 mmol/L glucose. At 3.3 mmol/L glucose, *cis* vaccenic acid (3.0 mmol/L) did not affect glucose oxidation. In contrast, 3.0 mmol/L oleic acid markedly inhibited glucose oxidation compared with the equimolar concentration of elaidic acid with 3.3 mmol/L glucose. Further, increased glucose oxidation was found with an increasing glucose concentration from 3.3 to 16.7 mmol/L for cells exposed to the *trans* isomers (*trans* vaccenic acid and elaidic acid), as well as cells not exposed to fatty acids. However, this differential influence on glucose oxidation with glucose concentrations of 3.3 and 16.7 mmol/L diminished or was absent when *cis* isomers were added.

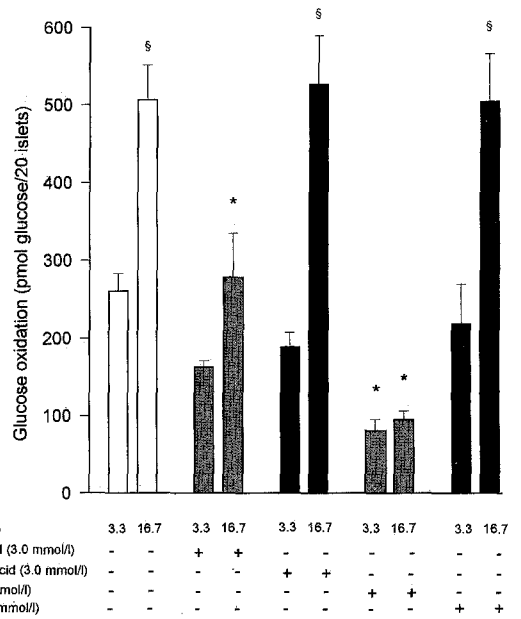

Fig 6. Effect of 3.0 mmol/L *cis* and *trans* fatty acids on glucose oxidation. Twenty mouse islets were incubated at 3.3 or 16.7 mmol/L glucose with or without the addition of 3.0 mmol/L fatty acids for 2 hours, and the $^{14}CO_2$ produced was measured. Samples were assayed in duplicate, and bars represent the mean ± SEM of 4 experiments. §$P < .05$ *v* low glucose (3.3 mmol/L) and otherwise identical incubation conditions. *$P < .05$ *v* the corresponding isomeric fatty acid (*trans* vaccenic or elaidic acid) at similar glucose level.

DISCUSSION

In the present study, we focused on the importance of the spatial configuration of fatty acids (*cis* and *trans* isomeric forms) to insulin secretion from isolated mouse islets. We found that the most common *trans* fatty acids (elaidic acid and *trans* vaccenic acid) stimulate insulin release more potently than the corresponding *cis* isomers (oleic acid and *cis* vaccenic acid). The effects of both isomers were glucose-dependent. In the presence of another insulin secretagogue, L-leucine, a differential action on insulin release was only observed for *cis* and *trans* vaccenic acid. Interestingly, we also demonstrated a differential influence of the isomeric fatty acids on islet glucose oxidation, which may explain, at least in part, the observed differences in the secretory response. The *cis* isomers of the fatty acids tested reduced islet glucose oxidation at high glucose, but the *trans* isomers did not.

According to Randle's theory,[22] an inverse relationship exists between the oxidation of glucose and fatty acids. A possible mechanism of this relationship is that β-oxidation results in an increased mitochondrial ratio of acetyl coenzyme A (CoA)/CoA, which may inhibit the pyruvate dehydrogenase complex and hexokinase[23,24] and thereby decrease glucose oxidation. In pancreatic rat islets, it has been shown that palmitate (C 16:0) slightly decreases glucose oxidation during a 2-hour incubation at high,[25] but not low, glucose concentrations.[10,25] However, during a 4-hour incubation of islets with palmitate at low glucose, a clear inhibition of glucose oxidation has been observed.[6]

Conget et al[10] have studied the impact of oleate on glucose oxidation at 8.3 mmol/L glucose in islets, and reported that no influence could be demonstrated. The apparent difference from our results might be ascribed to different experimental conditions, ie, the ratio between FFA and albumin was only half the ratio used in our study. Short-chain fatty acids (ie, octanoate C

Table 1. Effects of *Cis* and *Trans* Fatty Acids on L-Leucine–Stimulated Insulin Release

	Insulin Release After Incubation With Different Fatty Acids (3.0 mmol/L) (μU/mL)				
Additive	Control	*Cis* Vaccenic Acid	*Trans* Vaccenic Acid	Oleic Acid	Elaidic Acid
Glucose 4.0 mmol/L	20 ± 1	27 ± 2	24 ± 1	22 ± 1	28 ± 4
Glucose 16.7 mmol/L	104 ± 19‡	574 ± 35*	798 ± 62*†	584 ± 35*	833 ± 81*†
L-Leucine 5.0 mmol/L + glucose 4.0 mmol/L	137 ± 19‡	254 ± 25*	416 ± 42*†	314 ± 24*	339 ± 26*

NOTE. Single mouse islets were incubated for 60 minutes. Data are the mean ± SEM of 16-32 incubations.

*$P < .05$ *v* control (without fatty acids).

†$P < .05$ *v* corresponding isomeric fatty acid.

‡$P < .05$ *v* low glucose (4.0 mmol/L).

8:0) stimulate glucose oxidation at low glucose in rat islets but do not alter glucose oxidation at high glucose.[8] It is obvious that the influence of fatty acids on glucose oxidation depends on the type of fatty acid, glucose concentration, and duration of incubation.

To our knowledge, until now, no reports have compared the oxidation of isomeric fatty acids in rodent islets. However, in vivo in rats[26] and in rat heart mitochondria[27-29] and liver mitochondria,[30] *trans* fatty acids were oxidized at a slower rate compared with *cis* fatty acids. If similar conditions are present in rat β cells, a higher rate of oxidation of *cis* fatty acids would, via the Randle cycle, inhibit glucose oxidation to a greater extent than the corresponding *trans* isomers. According to the Randle cycle, fatty acids are expected to inhibit glucose oxidation, but this was only the case for *cis* fatty acids. Since production of acetyl CoA from glucose oxidation is about fourfold to eightfold higher than from palmitate oxidation,[8,25,31] palmitate may produce an insufficient amount of acetyl CoA to inhibit glucose oxidation. However, certain fatty acids may be oxidized at a higher rate, thereby causing an inhibition of glucose oxidation. In this context, it is noteworthy that *trans* vaccenic and elaidic acid tended to inhibit glucose oxidation at low glucose, where the production of acetyl CoA from glucose is relatively low (Fig 6).

An important mechanism underlying glucose-stimulated insulin secretion is that glucose oxidation in the β cell alters the adenosine triphosphate (ATP)/adenosine diphosphate ratio, leading to closure of the ATP-regulated K^+ channels, depolarization, and entry of Ca^{2+} through voltage-sensitive Ca^{2+} channels, which initiates exocytosis of insulin.[32,33] A negative interference with glucose oxidation is expected to decrease insulin secretion. Exposure of islets to fatty acids has shown that they may open Ca^{2+} channels in the plasma membrane,[9,10,34] as well as mobilize calcium from intracellular stores,[34] and eventually have a restoring effect on cytosolic Ca^{2+} through activation of Ca^{2+}-ATPases in the endoplasmic reticulum.[35] Thus, although fatty acids inhibit glucose oxidation and thereby diminish the intracellular calcium concentration, an additional effect on Ca^{2+} channels may counteract this effect on the Ca^{2+} concentration. This may result in increased insulin secretion despite decreased glucose oxidation. Interestingly, it has been demonstrated that the effects of oleic and elaidic acid on the ATP-regulated K^+ channel in islets are different: elaidic acid had no effect on the channel, but oleic acid opened it.[36] This may also contribute to the lower insulin-stimulatory effect of oleic acid compared with elaidic acid. L-Leucine–stimulated insulin release is accompanied by closure of the ATP-regulated K^+ channels and depolarization of the cell membrane,[37,38] increased malonyl CoA content,[14] as well as increased phosphoinositide hydrolysis,[39,40] all features of the stimulus-secretion coupling of glucose-induced insulin release. Keeping this in mind, it was expected that the differential effects of *cis* and *trans* isomeric fatty acids observed with glucose also would be present for L-leucine. However, a differential effect on β-cell function could be detected for *cis* and *trans* vaccenic acid, but not for oleic and elaidic acid. One explanation could be that the position isomers of fatty acids (*cis* and *trans* vaccenic acid *v* oleic and elaidic acid) interfere with the metabolism of L-leucine to acetyl CoA, in different ways.

Long-chain fatty acids are poorly soluble in most aqueous media, but with the addition of albumin it is possible to dissolve more FFAs. Most previous studies on the effect of fatty acids on insulin secretion have used albumin concentrations of 0.5% to 2%. The concentration of albumin in human plasma is 3% to 4%, and we have consequently chosen 3% albumin in our experiments. Hence, we are able to dissolve a high amount of fatty acids (3 to 4 mmol/L) and find a maximal stimulation on insulin release at 2.0 to 3.0 mmol/L of the fatty acids studied. This corroborates findings by Prentki et al,[14] who showed that palmitic acid markedly potentiates insulin secretion from HIT cells at a palmitate to albumin ratio (millimolars to percent weight per volume) of 1. At concentrations greater than 3.0 mmol/L fatty acid, insulin secretion declines. At this concentration, the solutions were turbid, probably because of formation of large amounts of aggregated fatty acids[41,42] as a result of surpassing the binding capacity for albumin. The resultant increased free amount of fatty acids may act negatively on membrane proteins or disrupt calcium or proton gradients.[43-45] Albumin contains several binding sites, to which fatty acids with a different chain length and degree of saturation bind with varying affinity.[46] It has been demonstrated that the free (unbound) fatty acid plays the major role in potentiation of insulin secretion.[9,14] Thus, the observed differential effect of *cis* and *trans* isomers on insulin release may simply be ascribed to different binding affinities of *cis* and *trans* fatty acids to BSA, and consequently, different amounts of unbound fatty acids. Figure 1 shows that the maximal stimulation of insulin secretion is obtained at about 2.0 mmol/L for both *cis* and *trans* vaccenic acid, with a similar shape for the concentration-response curve. This may indicate that the higher insulinotropic effect of *trans* vaccenic acid is not caused by a higher amount of free *trans* versus *cis* vaccenic acid, since this would tend to shift the concentration-response curve to the left. The same can be argued for oleic and elaidic acid.

The amount of *trans* fatty acids in the plasma of non-obese persons constitutes about 0.5% to 2% of total fatty acids depending on the intake of *trans* fatty acids,[47,48] which corresponds to a plasma concentration of about 0.01 mmol/L *trans* fatty acids. At this concentration, we did not find any influence on insulin secretion from islets. However, in vivo, *trans* fatty acid may well act in synergy with the more prevalent circulating fatty acids, eg, palmitate and oleate.

In conclusion, we have demonstrated that *trans* isomers of fatty acids potentiate glucose-stimulated insulin secretion more than *cis* isomers of identical carbon chain length. The mechanism for this differential response may be linked to a differential effect on glucose oxidation. Our findings suggest that the spatial configuration of fatty acids is important to the regulation of β-cell secretion in the mouse. Whether the long-term effects of *trans* fatty acids to increase insulin secretion result eventually in β-cell damage or an overall negative pattern of metabolism remains to be elucidated.

ACKNOWLEDGMENT

The technical expertise of Jytte Søholt, Dorthe Rasmussen, and Kirsten Eriksen is gratefully acknowledged. We thank Hans Kofod, Novo Nordisk, for the generous gift of antibody for the insulin assay.

REFERENCES

1. Chen YDI, Golay A, Swislocki ALM, et al: Resistance to insulin suppression of plasma free fatty acid concentrations and insulin stimulation of glucose uptake in noninsulin-dependent diabetes mellitus. J Clin Endocrinol Metab 64:17-21, 1987

2. Gordon ES: Non-esterified fatty acids in the blood of obese and lean subjects. Am J Clin Nutr 8:740-747, 1960

3. Lee Y, Hirose H, Ohneda M, et al: Beta-cell lipotoxicity in the pathogenesis of non–insulin-dependent diabetes mellitus of obese rats: Impairment in adipocyte–beta-cell relationships. Proc Natl Acad Sci USA 91:10878-10882, 1994

4. Zhou YP, Grill V: Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose induced insulin secretion and biosynthesis through a glucose fatty acid cycle. J Clin Invest 93:870-876, 1994

5. Milburn JL, Hirose H, Lee Y, et al: Pancreatic beta-cells in obesity. Evidence for induction of functional, morphologic, and metabolic abnormalities by increased long chain fatty acids. J Biol Chem 270:1295-1299, 1995

6. Elks ML: Chronic perifusion of rat islets with palmitate suppresses glucose-stimulated insulin release. J Endocrinol 133:208-214, 1993

7. Opara EC, Garfinkel M, Hubbard VS, et al: Effect of fatty acids on insulin release: Role of chain length and degree of unsaturation. Am J Physiol 266:E635-E639, 1994

8. Zhou YP, Ling Z, Grill V: Inhibitory effects of fatty acids on glucose-regulated β-cell function: Association with increased islet triglyceride stores and altered effect of fatty acid oxidation on glucose metabolism. Metabolism 45:981-986, 1996

9. Warnotte C, Gilon P, Nenquin M, et al: Mechanisms of the stimulation of insulin release by saturated fatty acids. A study of palmitate effects in mouse beta-cells. Diabetes 43:703-711, 1994

10. Conget I, Rasschaert J, Sener A, et al: Secretory, biosynthetic, respiratory, cationic, and metabolic responses of pancreatic islets to palmitate and oleate. Biochem Med Metab Biol 51:175-184, 1994

11. Balasse EO, Ooms HA: Role of plasma free fatty acids in the control of insulin secretion in man. Diabetologia 9:145-151, 1973

12. Zhou YP, Berggren P, Grill V: A fatty acid–induced decrease in pyruvate dehydrogenase activity is an important determinant of beta-cell dysfunction in the obese diabetic db/db mouse. Diabetes 45:580-586, 1996

13. Garfinkel M, Lee S, Opara EC, et al: Insulinotropic potency of lauric acid: A metabolic rationale for medium chain fatty acids (MCF) in TPN formulation. J Surg Res 52:328-333, 1992

14. Prentki M, Vischer S, Glennon MC, et al: Malonyl-CoA and long chain acyl-CoA esters as metabolic coupling factors in nutrient-induced insulin secretion. J Biol Chem 267:5802-5810, 1992

15. Stein DT, Stevenson BE, Chester MW, et al: The insulinotropic potency of fatty acids is influenced profoundly by their chain length and degree of saturation. J Clin Invest 100:398-403, 1997

16. Christiansen E, Schneider S, Palmvig B, et al: Intake of a diet high in *trans* monounsaturated fatty acids or saturated fatty acids. Diabetes Care 20:881-887, 1997

17. Emken EA: Nutrition and biochemistry of *trans* and positional fatty acid isomers in hydrogenated oils. Annu Rev Nutr 4:339-376, 1984

18. Becker W: Intake of *trans* fatty acids in the Nordic countries. Scand J Nutr/Naringsforskning 40:16-18, 1996

19. Lacy PE, Kostianovsky M: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 16:35-39, 1967

20. Gregersen S, Thomsen JL, Brock B, et al: Endothelin-1 stimulates insulin secretion by direct action on the islets of Langerhans in mice. Diabetologia 39:1030-1035, 1996

21. Pedersen O, Hjøllund E, Lindskov HO: Insulin binding and action on fat cells from young healthy females and males. Am J Physiol 243:E158-E167, 1982

22. Randle PJ, Garland PB, Hales CN, et al: The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbance of diabetes mellitus. Lancet 1:785-789, 1963

23. Weber G, Convery HJH, Lea MA, et al: Feedback inhibition of key glycolytic enzymes in liver: Action of free fatty acids. Science 154:1357-1360, 1966

24. Randle PJ, Priestman DA, Mistry SC, et al: Glucose fatty acid interactions and the regulation of glucose disposal. J Cell Biochem 55S:1-11, 1994 (suppl)

25. Alcazar O, Qiu-yue Z, Giné E, et al: Stimulation of islet protein kinase C translocation by palmitate requires metabolism of the fatty acid. Diabetes 46:1153-1158, 1997

26. Anderson RL, Coots RH: The catabolism of the geometric isomers of uniformly ^{14}C-labeled delta 9-octadecenoic acid and uniformly ^{14}C-labeled delta 9,12-octadecadienoic acid by the fasting rat. Biochim Biophys Acta 144:525-531, 1967

27. Lanser AC, Emken EA, Ohlrogge JB: Oxidation of oleic and elaidic acids in rat and human heart homogenates. Biochim Biophys Acta 875:510-515, 1986

28. Lawson LD, Kummerow FA: Beta-oxidation of the coenzyme A esters of vaccenic, elaidic and petroselaidic acids by rat heart mitochondria. Lipids 14:501-503, 1978

29. Lawson LD, Kummerow FA: Beta-oxidation of the coenzyme A esters of elaidic, oleic and stearic acids and their full-cycle intermediates by rat heart mitochondria. Biochim Biophys Acta 573:245-254, 1979

30. Lawson LD, Holman RT: Beta-oxidation of the geometric and positional isomers of octadecenoic acid by rat heart and liver mitochondria. Biochim Biophys Acta 665:60-65, 1981

31. Malaisse WJ, Malaisse-Lagae F, Sener A, et al: Participation of endogenous fatty acids in the secretory activity of the pancreatic B-cell. Biochem J 227:995-1002, 1985

32. Prentki M: New insights into pancreatic beta-cell metabolic signaling in insulin secretion. Eur J Endocrinol 143:272-286, 1996

33. Malaisse WJ, Sener A, Herchuelz A, et al: Insulin release: The fuel hypothesis. Metabolism 28:373-386, 1979

34. Metz SA: Exogenous arachidonic acid promotes insulin release from intact or permeabilized rat islets by dual mechanisms. Putative activation of Ca^{2+} mobilization and protein kinase C. Diabetes 37:1453-1469, 1998

35. Deeney JT, Thornheim K, Korchak HM, et al: Acyl-CoA esters modulate intracellular Ca^{2+} handling by permeabilized clonal pancreatic beta-cells. J Biol Chem 267:19840-19845, 1992

36. Müller M, Szewczyk A, Weille JR, et al: ATP-sensitive K^+ channels in insulinoma cells are activated by nonesterified fatty acids. Biochemistry 31:4656-4661, 1992

37. Henquin JC, Meissner HP: Effects of amino acids on membrane potential and 86Rb+ fluxes in pancreatic beta-cells. Am J Physiol 240:E245-E252, 1981

38. Flatt PR, Barnett CR, Shibier O, et al: Direct and indirect actions of nutrients in the regulation of insulin secretion from the pancreatic beta-cells. Proc Nutr Soc 50:559-566, 1991

39. Zawalich WS: Time-dependent potentiation of insulin release induced by alpha-ketoisocaproate and leucine in rats: Possible involvement of phosphoinositide hydrolysis. Diabetologia 31:435-442, 1988

40. Best L, Malaisse WJ: Phospholipids and islet function. Diabetologia 25:299-305, 1983

41. Vorum H, Brodersen R: Adsorption and physical state of

medium- and long-chain fatty acids in neutral aqueous buffer solution. Chem Phys Lipids 74:43-48, 1994

42. Brodersen R, Vorum H, Skriver E, et al: Serum albumin binding of palmitate and stearate. Multiple binding theory for insoluble ligands. Eur J Biochem 182:19-25, 1989

43. Cistola DP, Hamilton JA, Jackson D, et al: Ionization and phase behavior of fatty acids in water: Application of the Gibbs phase rule. Biochemistry 27:1881-1888, 1988

44. Corr PB, Gross RW, Sobel BE: Amphipathic metabolites and membrane dysfunction in ischemic myocardium. Circ Res 55:135-154, 1984

45. Pande SV, Mead JF: Inhibition of enzyme activities by free fatty acids. J Biol Chem 243:6180-6185, 1968

46. Spector AA, Fletcher JE, Ashbrook JD: Analysis of long-chain free fatty acid binding to bovine serum albumin by determination of stepwise equilibrium constants. Biochemistry 10:3229-3232, 1971

47. Nestel P, Noakes M, Belling B, et al: Plasma lipoprotein lipid and Lp(a) changes with substitution of elaidic acid or oleic acid in the diet. J Lipid Res 33:1029-1036, 1992

48. Siguel EN, Lerman RH: *Trans*-fatty acid patterns in patients with angiographically documented coronary artery disease. Am J Cardiol 71:916-920, 1993