Long-Term Exposure of INS-1 Cells to *Cis* and *Trans* Fatty Acids Influences Insulin Release and Fatty Acid Oxidation Differentially

K.K. Alstrup, B. Brock, and K. Hermansen

The importance of elevated levels of fatty acids in the pathogenesis of the deteriorated β-cell function present in type 2 diabetes has been established. Long-term exposure of the β-cell to high levels of fatty acids causes enhanced insulin secretion at low glucose (basal insulin release), while glucose-stimulated insulin secretion (GSIS) is decreased or unchanged. We have previously demonstrated that the spatial configuration of fatty acids (*cis* and *trans* isomers) is of importance for the acute impact on the β-cell function. In this study we aimed to elucidate whether the spatial configuration also influenced β-cell function after long-term exposure. Thus, we compared the effect of 3 days culture of INS-1 cells with *cis* (*cis* C 18:1-11) and *trans* vaccenic acid (*trans* C 18:1-11), as well as oleic (*cis* C 18:1-9) and elaidic acid (*trans* C 18:1-9), on basal and glucose-stimulated insulin release. All fatty acids tested increased basal insulin release; however, a significantly lower basal insulin release was demonstrated for cells cultured with 0.3 to 0.4 mmol/L *trans* vaccenic acid compared to equimolar levels of the *cis* isomer. GSIS was not changed by *cis* or *trans* vaccenic acid or by oleic acid, whereas it was stimulated by 0.3 to 0.4 mmol/L elaidic acid. The mechanisms behind the fatty acid–induced changes in the β cells have been linked to changes in glucose and fatty acid oxidation. We demonstrated an increased fatty acid oxidation in β cells after long-term exposure to all of the tested fatty acids. Interestingly, both *trans* isomers (*trans* vaccenic and elaidic acid) induced higher fatty acid oxidation than the *cis* isomers (*cis* vaccenic and oleic acid, respectively). No changes in glucose oxidation were found when INS-1 cells were cultured with either of the fatty acids. The increased fatty acid oxidation was associated with an increased content of carnitine palmitoyltransferase I (CPT-I) mRNA, but no difference in the content of CPT-I mRNA to the different fatty acids was found. Insulin mRNA expression in β cells was not affected by the fatty acids. In conclusion, we have demonstrated that the pathological changes in insulin secretion from INS-1 cells to long-term culture with elevated levels of fatty acids are more pronounced for the *cis* (*cis* vaccenic acid and oleic acid) rather than the *trans* isomers (*trans* vaccenic acid and elaidic acid). We suggest that this, at least in part, may be explained by a lower fatty acid oxidation in cells cultured with the *cis* compared to the *trans* fatty acid isomers. Apparently, the difference in fatty acid oxidation was not caused by an increased induction of CPT-I mRNA, nor by changes in glucose oxidation or insulin mRNA in β cells chronically exposed to the fatty acids.

FATTY ACIDS play an important role in islet cell function. They are known to stimulate insulin secretion acutely both in vitro[1-3] and in vivo.[4] In contrast, long-term exposure to fatty acids in humans and rodents causes an impaired glucose-stimulated insulin secretion (GSIS) in vitro[1,5-11] and in vivo,[1,5,8,11] whereas basal insulin secretion (BIS) is increased[7,8]; these changes resemble those found in type 2 diabetes.

The insulinotropic effect appears to decrease with the degree of unsaturation at high glucose levels,[12] while it has been found to increase in the presence of low glucose levels.[13]With increasing chain length the acute insulinotropic effect of fatty acids is enhanced.[12,14] Also, the spatial configuration (*cis*/*trans* form) of fatty acids seems to be of importance.[15] *Trans* fatty acids are unsaturated fatty acids with the carbon moieties on the 2 sides of the double bond providing a straight, closely packed configuration, while *cis* isomers have a bent configuration. Palmitelaidic acid (*trans* C 16:1-9) tended to be more insulinotropic than the *cis* isomer in the perfused rat pancreas.[12] In addition, we demonstrated that acute exposure of isolated mouse islets to *trans* fatty acids induces higher insulin release than the corresponding *cis* isomers.[14]

Recent reports indicate that a fuel "cross-talk" between fatty acids and glucose is probably operative in the pancreatic β cell; that is, changes in glucose and fatty acid oxidation may be involved in the deteriorated β cell function following chronic exposure to elevated levels of fatty acids.[16] Long-term exposure of β cells to fatty acids enhances fatty acid oxidation,[17,18] which seems to be related to increased expression and activity of carnitine palmitoyltransferase I (CPT-I).[17] Apparently, fatty acids directly induce transcriptional activation of the *CPT-I* gene by a mechanism yet unknown. In addition, glucose oxidation is influenced by chronic exposure of β cells to fatty acids, with most studies finding it attenuated.[1,7,11,19] Consequently, fatty acids may shift the metabolism of lipids and glucose into a relatively more pronounced fatty acid oxidation compared to glucose oxidation, which may be coupled to impairment in insulin secretion.

Under normal conditions, a delicate balance exists between insulin production and secretion. With prolonged exposure of β cells to fatty acids, a balance may still be obtained, although at a different level. There have been contrasting reports on how

From the Department of Medicine, Randers Centralsygehus, Randers, Denmark; Department of Clinical Pharmacology, Aarhus Kommunehospital, Aarhus University Hospital; and the Department of Endocrinology and Metabolism, Aarhus Amtssygehus, Aarhus University Hospital, Aarhus, Denmark.

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Address reprint requests to K.K. Alstrup, MD, PhD, Randels Centralsygehus, Deaprtment of Medicine, Skovlyvej 1, Randers 8900, Denmark.

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fatty acids affect the expression of insulin mRNA in islets: some find it increased[20] and others decreased.[21,22] However, long-term exposure of rat islets to increased levels of fatty acids blunted the proinsulin biosynthesis[7,20] and pancreatic insulin content.[20,22]

The aim of the present study was to determine whether the differential effect on acute insulin release to isomeric fatty acids, ie, *cis* (*cis* C 18:1-11) and *trans* vaccenic acid (*trans* C 18:1-11) as well as oleic (*cis* C 18:1-9) and elaidic acid (*trans* C 18:1-9), was also operative after long-term exposure. Furthermore, we examined if such an effect is related to changes in glucose or fatty acid oxidation, and CPT-I or insulin mRNA expression.

MATERIALS AND METHODS

Culturing of Cells

INS-1 cells (passages below 68) were grown in monolayer and cultured as described previously[23] in RPMI 1640 medium containing 10 mmol/L glucose supplemented with 10 mmol/L HEPES, 10% heat-inactivated fetal calf serum, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 50 μmol/L β-mercaptoethanol, 100 IU/mL penicillin, and 100 μg/mL streptomycin (all Life Technologies, Paisley, Scotland) in an humified atmosphere (5%CO_2, 95% air). Three days prior to secretion and oxidation studies, the cells were cultured in medium containing 5.0 mmol/L glucose and 0.5% bovine serum albumin (BSA, defatted) (Boehringer Mannheim, Mannheim, Germany) with or without addition of different fatty acids. To maintain a constant concentration of glucose and fatty acids, the medium was changed every day. In experiments measuring insulin or CPT-1 mRNA content, cells were preincubated in culture medium containing 5.0 mmol/L glucose for 24 hours. Cells were then cultured for another 24 hours in medium containing 5.0 mmol/L glucose, 0.5% BSA with or without different fatty acids.

Secretion Studies

For secretion studies cells (0.2 mio cells/well) were plated in 24-well plates (NUNC A/S, Roskilde, Denmark) and cultured as described above. On the day of experiment, the media was changed to a HEPES-buffered medium containing 3.3 mmol/L glucose and preincubated for 20 minutes. The HEPES-buffered medium contained 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) (all Sigma Chemical Co, St Louis, MO), and 0.1% BSA. The buffer was then changed to a similar buffer containing 3.3 mmol/L glucose or 16.7 mmol/L glucose and the cells were incubated for another 60 minutes at 37°C. An aliquot of the medium was collected and stored for later determination of insulin.

Protein Measurement

After performing secretion studies, the cells were lysed in 0.1N NaOH and total protein was determined by the Bradford method[24] using Bio-Rad Protein assay Dye reagent (Bio-Rad Laboratories, Hercules, CA).

Preparation of Fatty Acids

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 (Sigma) supplemented with distilled H_2O to a final concentration of 100 mmol/L. This solution was stored at −20°C. On the day of the experiment, an appropriate volume of hot (80 to 90°C) stock solution was added slowly and during continuous agitation to the culturing medium containing 0.5% BSA. The amount of fatty acids added was in surplus relative to the number of cells in order to keep constant the concentration of fatty acids during the incubation.

Samples were removed from the wells after 24 hours of culturing and fatty acid concentration was determined using a nonesterified fatty acids (NEFA) kit (Wako Chemicals, Neuss, Germany). There was no change in the fatty acid concentration during the culturing period.

Insulin Assay

The concentration of insulin was determined by radioimmunoassay with a guinea-pig antiporcine insulin antibody PNILGP4 (Novo Nordisk, Bagsvaerd, Denmark) and mono-^{125}I- (Tyr A14)-labeled human insulin (Novo Nordisk) as tracer and rat insulin (Novo Nordisk) as standard. Free and bound radioactivity were separated using ethanol. Inter- and intra-assay variation was between 5% and 10%.

Glucose Oxidation

Glucose oxidation in INS-1 cells was determined by measuring the formation of $^{14}CO_2$ from [U-^{14}C]glucose (Amersham Life Science)[25] After culture in different media, cells were trypsinized and preincubated as suspension in RPMI 1640 containing 10 mmol/L HEPES, 0.5% BSA, and 5.0 mmol/L glucose for 1 hour at 37°C. Cells were then washed and resuspended in the same medium containing different glucose concentrations and [U-^{14}C]glucose (0.5 μCi per sample), gassed with O_2/CO_2 (95%/5%), and incubated for 2 hours at 37°C. The CO_2 collection system consisted of 2 connected tubes creating an airtight chamber with the cell suspensions in the bottom tube and a Whatman GF/C filter (Whatman International, Maidstone, England) containing 200 μL 25% (wt/vol) phenethylamine (Merck, Darmstadt, Deutchland) in methanol in the upper tube.[26] The metabolic reaction was stopped by injecting 150 μL of 10% trichloroacetic acid (TCA) through the upper tube and the tubes were immediately pushed together closing the injection hole. The tubes were left at room temperature for 5 hours to allow the produced $^{14}CO_2$ to absorb the phenethylamine. Blank incubations without cells were treated similarly. Radioactivity was determined by a liquid scintillation counter. The recovery of $^{14}CO_2$ as assessed with $NaH^{14}CO_3$ was 82% ± 1% (n = 12). Glucose oxidation was expressed in nanomoles of oxidized glucose per 1.5 mio cells during 2 hours incubation.

Fatty Acid Oxidation

Fatty acid oxidation was determined by measuring the production of $^{14}CO_2$ from [U-^{14}C] palmitic acid (NEN, Boston, MA) using the same experimental design as described for glucose oxidation except that cells were incubated in RPMI 1640 medium supplemented with 10 mmol/L HEPES, 0.5% BSA, 0.4 mmol/L [U-^{14}C] palmitate (0.2 μCi per sample), and various glucose concentrations. After the metabolic reaction was stopped by injection of 10% TCA, samples were left at room temperature to collect $^{14}CO_2$ overnight. Blank samples without cells were treated similarly. Palmitate oxidation was expressed in picomoles oxidized palmitate per 1.5 mio cells during 2 hours incubation.

Insulin and CPT-1 mRNA Analysis

Total RNA was extracted using Trizol (Life Technologies). This was followed by phenol-chloroform extraction and precipitation with alcohol.[26] RNA was meansured spectrophotometrically and the quality was tested by a 1% agarose-gel electrophoresis followed by staining with ethidium bromide, which showed no signs of DNA contamination or degradation. RNA samples (10 μg) were denaturated in dimethyl sulfoxide (DMSO)/glyoxal at 60°C for 30 minutes, subjected to 1% agarose-gel electrophoresis,[27] and transferred to Hybond N membranes (HYBOND N, Amersham, England) by capillary elution. CPT-I mRNA was detected by α-[^{32}P]-labeled antisense RNA probe transcribed from the linarized plasmid pBSK-CPTIr containing the cDNA

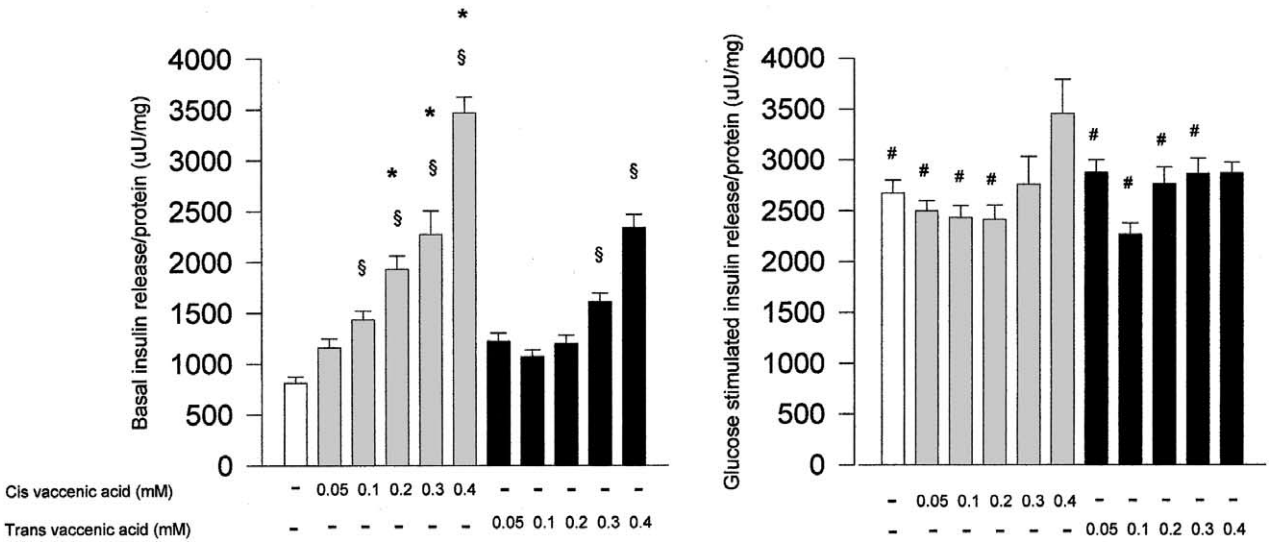

Fig 1. (A) Insulin release from cells incubated for 1 hour at 3.3 mmol/L glucose (basal insulin release) relative to total cell protein. (B) Insulin release from cells incubated for 1 hour at 16.7 mmol/L glucose (GSIS) relative to total cell protein. Cells were precultured for 3 days with either no fatty acids (white bars), *cis* vaccenic acid (0.05 to 0.4 mmol/L) (grey bars), or *trans* vaccenic acid (0.05 to 0.4 mmol/L) (black bars). Each bar represents the mean ± SEM of 30 to 36 single incubations. §$P < .05$ for comparisons between fatty acids and control. *$P < .05$ for comparisons between *cis* and *trans* vaccenic acid at equimolar levels at identical incubations. #$P < .05$ for comparisons between basal insulin release and GSIS at identical culturing conditions.

for rat liver CPT-I using the Promega in vitro Transcription System (Promega Corp, Madison, WI). Membranes were prehybridized in 50% formamide, 5X sodiumchlorid disodiumdihydrogenphosphate EDTA (SSPE), 2X Denhardt's solution, 0.1% sodium dodecyl sulfate (SDS), and 1.04 mg/mL milk powder at 60°C for 4 hours followed by hybridization with the RNA probe (1 to 2 $\times 10^6$ cpm/mL) at 60°C overnight. Membranes were washed 3 times in 0.1X SSC, 0.1% SDS at 65°C for 30 minutes. Insulin mRNA was detected by α-[^{32}P]-labeled rat insulin II cDNA prepared by Nick Translation System (Promega) and β-actin mRNA by α-[^{32}P]-labeled cDNA probe generated from human β-actin cDNA (Clontec Laboratories, Palo Alto, CA) by random priming. Hybridization buffer for detection of β-actin and insulin mRNA was as described for RNA probe, and hybridization was performed overnight at 42°C, followed by washing 3 times for 30 minutes each in 1X SSPE, 0.1% SDS at 55°C. The autoradiograms were analyzed by densitometric scanning and mRNA values were normalized to those of β-actin mRNA.

Statistical Analysis

For statistical comparisons, analysis of variance (ANOVA) was used followed by the Newman-Keuls test. Results are expressed as means ± SEM. Statistical significance was detected at $P < .05$.

RESULTS

Effects of Exposure of INS-1 Cells to Cis and Trans Vaccenic Acid on BIS and GSIS

The effect of 3 days culture of INS-1 cells in the presence of 5.0 mmol/L glucose and increasing concentrations (0.05 to 0.4 mmol/L) of *cis* or *trans* vaccenic acid on subsequent basal insulin release (1-hour incubation at 3.3 mmol/L glucose) is shown in Fig 1A. With increasing concentration of *cis* vaccenic acid (0.1 to 0.4 mmol/L), a dose-dependent increase in basal insulin release was observed ($P < .05$). Culture with *trans* vaccenic acid only resulted in increased basal insulin release at concentrations of 0.3 and 0.4 mmol/L ($P < .05$). It shold be noted that 0.2 to 0.4 mmol/L *cis* vaccenic acid caused higher basal insulin release (40% to 60%) than did equimolar concentrations of *trans* vaccenic acid ($P < .05$).

The effect of culturing cells with *cis* or *trans* vaccenic acid (0.05 to 0.4 mmol/L) on GSIS at 16.7 mmol/L glucose is shown in Fig 1B. The fatty acids did not change the GSIS compared to control, and there were no significant differences in GSIS between the 2 isomers.

Figure 1 (A and B) derives from the same experiments, allowing us to compare the results directly. It can be seen that cells cultured with 0.5% BSA alone are glucose-sensitive since insulin secretion to 16.7 mmol/L glucose is increased about 3 times compared to basal insulin release. Furthermore, cells cultured with 0.3 to 0.4 mmol/L *cis* vaccenic acid have elevated basal insulin release, reaching a level comparable to the GSIS level. However, GSIS is significantly higher than basal insulin release ($P < .05$) when cells are cultured at the lower concentrations of 0.05 to 0.2 mmol/L *cis* vaccenic acid. With the exception of a concentration of 0.4 mmol/L, *trans* vaccenic acid elicits higher GSIS than basal insulin release ($P < .05$).

The differential effect on insulin secretion of culturing with *cis* or *trans* vaccenic acid was only present at normal glucose levels (5.0 mmol/L glucose). When the glucose concentration was increased to 11.0 to 16.7 mmol/L, *cis* and *trans* vaccenic acid influenced BIS and GSIS to the same extent (data not shown).

Effects of Exposure of INS-1 Cells to Oleic and Elaidic Acid on BIS and GSIS

Cells were cultured for 3 days at 5.0 mmol/L glucose and increasing concentrations (0.05 to 0.4 mmol/L) of oleic and

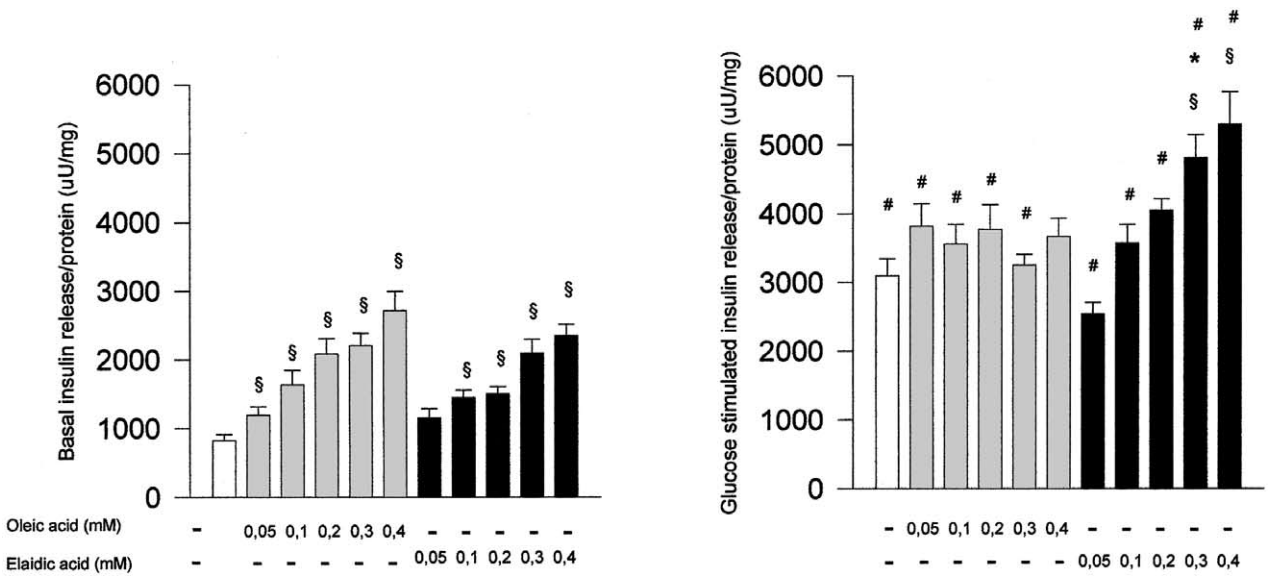

Fig 2. (A) Insulin release from cells incubated for 1 hour at 3.3 mmol/L glucose (basal insulin release) relative to total cell protein. (B) Insulin release from cells incubated for 1 hour at 16.7 mmol/L glucose (GSIS) relative to total cell protein. Cells were precultured for 3 days with either no fatty acids (white bars), oleic acid (0.05 to 0.4 mmol/L) (grey bars), or elaidic acid (0.05-0.4 mmol/L) (black bars). Each bar represents the mean ± SEM of 30 to 36 single incubations. §$P < .05$ for comparisons between fatty acids and control. *$P < .05$ for comparisons between oleic and elaidic acid at equimolar levels at identical incubations. #$P < .05$ for comparisons between basal insulin release and GSIS at identical culturing conditions.

elaidic acid followed by estimation of BIS and GSIS. As can be seen from Fig 2A, a dose-dependent increase in BIS was found with increasing concentrations of both fatty acids. Oleic and elaidic acid enhanced BIS at concentrations of 0.05 to 0.4 mmol/L and 0.1 to 0.4 mmol/L ($P < .05$), respectively. There was no difference in the ability of the 2 fatty acids to elevate basal insulin secretion.

No effect on GSIS occurred in response to increasing concentrations of oleic acid (0.05 to 0.4 mmol/L) compared to cells cultured with 0.5% BSA alone (Fig 2B). In contrast, culturing cells with increasing concentrations of elaidic acid elicited a dose-dependent increase in GSIS, being higher at 0.3 to 0.4 mmol/L elaidic acid than the control ($P < .05$). A significantly higher GSIS than BIS was observed at 0.05 to 0.3 mmol/L oleic acid ($P < .05$). At 0.4 mmol/L oleic acid, BIS and GSIS did not differ significantly. For elaidic acid (0.05 to 0.4 mmol/L), a 2-fold increase in insulin release was found when cells were stimulated with 16.7 mmol/L glucose compared to 3.3 mmol/L glucose ($P < .05$).

When glucose was increased to 11.0 to 16.7 mmol/L, the influence of oleic and elaidic acid on BIS or GSIS was similar (data not shown).

Influence of Long-Term Culture With Isomeric Fatty Acids on Fatty Acid Oxidation

The rate of palmitate oxidation at both low and high glucose was significantly increased for cells cultured with either fatty acid compared to cells cultured with 0.5% BSA alone ($P < .05$) (Table 1). The increase in palmitate oxidation was 2- to 4.5-fold compared to cells cultured in 0.5% BSA alone. For all culture conditions (with or without fatty acids) palmitate oxidation was significantly higher in the presence of low glucose (3.3 mmol/L) compared to high glucose (16.7 mmol/L) ($P < .05$).

Interestingly, palmitate oxidation in cells cultured with *trans* vaccenic acid (0.4 mmol/L) was increased by 30% at low glucose ($P < .05$) compared to cells cultured with *cis* vaccenic acid (0.4 mmol/L). The same tendency was found for palmitate oxidation at high glucose, although this did not reach statistical significance. In cells cultured with elaidic acid (0.4 mmol/L),

Table 1. Effects of Culture With Isomeric Fatty Acids on Palmitate Oxidation

Culture Conditions	Palmitate Oxidation (pmol/1.5 mio cells during 2-h incubation): Glucose in Incubation Medium (mmol/L)	
	3.3	16.7
Control (0.5% BSA)	297 ± 54	122 ± 25*
Cis vaccenic acid (0.4 mmol/L)	630 ± 31†	265 ± 23*†
Trans vaccenic acid (0.4 mmol/L)	813 ± 56†‡	375 ± 56*†
Oleic acid (0.4 mmol/L)	850 ± 41†	313 ± 24*†
Elaidic acid (0.4 mmol/L)	1272 ± 63†‡	546 ± 41*†‡

NOTE. Cells were cultured for 3 days at 5.0 mmol/L glucose in the presence of 0.5% BSA alone or with addition of 0.4 mmol/L isomeric fatty acids. Then cells were incubated with labeled palmitate and 3.3 or 16.7 mmol/L glucose for 2 hours and the production of $^{14}CO_2$ was measured. Data are the mean ± SEM of 8 or 9 separate incubations.

*$P < .05$ *v* low glucose (3.3 mmol/L) and otherwise identical incubation condition.

†$P < .05$ *v* control (0.5% BSA) at similar glucose concentration.

‡$P < .05$ *v* the corresponding isomeric fatty acid

Table 2. Effects of Culture With Isomeric Fatty Acids on Glucose Oxidation

Culture Conditions	Glucose Oxidation (nmol/1.5 mio cells during 2-h incubation): Glucose in Incubation Medium (mmol/L)	
	3.3	16.7
Control (0.5% BSA)	2.25 ± 0.46	5.50 ± 0.70*
Cis vaccenic acid (0.4 mmol/L)	1.93 ± 0.23	5.50 ± 0.38*
Trans vaccenic acid (0.4 mmol/L)	2.13 ± 0.20	5.63 ± 0.35*
Oleic acid (0.4 mmol/L)	1.89 ± 0.09	5.97 ± 0.31*
Elaidic acid (0.4 mmol/L)	1.80 ± 0.16	6.03 ± 0.66*

NOTE. Cells were cultured for 3 days at 5.0 mmol/L glucose in the presence of 0.5% BSA alone or with addition of 0.4 mmol/L isomeric fatty acids. Then cells were incubated with labeled palmitate and 3.3 or 16.7 mmol/L glucose for 2 hours and the production of $^{14}CO_2$ was measured. Data are the mean ± SEM of 12 separate incubations.

*$P < .05$ *v* low glucose (3.3 mmol/L) and otherwise identical incubation condition

palmitate oxidation in the presence of 3.3 or 16.7 mmol/L glucose was 50% and 74% higher, respectively, compared to cells cultured with oleic acid (0.4 mmol/L) ($P < .05$).

Influence of Long-Term Culture With Isomeric Fatty Acids on Glucose Oxidation

Glucose oxidation was significantly higher at high glucose (16.7 mmol/L) rather than at low glucose (3.3 mmol/L) under the same culturing conditions ($P < .05$) (Table 2). No difference in glucose oxidation was seen at the same glucose levels irrespective of addition of fatty acids or not.

CPT-I and Insulin mRNA Expression in INS-1 Cells Treated With Isomeric Fatty Acids

INS-1 cells were cultured for 24 hours at varying concentrations (0.05 to 0.4 mmol/L) of *cis* and *trans* vaccenic acids. As can be seen in Fig 3, both *cis* and *trans* vaccenic acid (0.05 to 0.4 mmol/L) caused a dose-dependent increase in the CPT-I mRNA expression. The maximal induction of CPT-I mRNA was about 6-fold higher than control for both fatty acids. There was no difference regarding the impact of the 2 isomeric fatty acids on CPT-I mRNA.

The insulin mRNA content was not changed irrespective of whether the fatty acid–treated cells were compared to control or to cells treated with the corresponding isomeric fatty acid.

Similar observations were made for cells treated with oleic and elaidic acid (0.05 to 0.4 mmol/L) (Fig 4). A dose-dependent increase in CPT-1 mRNA content in response to oleic and elaidic acid compared to control was found. Furthermore, equimolar concentrations of oleic acid and elaidic acid induced a similar content of CPT-I mRNA. No changes were seen in insulin mRNA content in INS-1 cells cultured with or without fatty acids.

DISCUSSION

In the present study we demonstrate that long-term exposure to isomeric *trans* and *cis* fatty acids differentially influences the secretory capacity of the β cell. In addition, the fatty acid oxidation seems to be affected by the spatial configuration of the fatty acids. Apparently, this could not be ascribed to a differential influence of the isomeric fatty acids on the transcription of CPT-I. We did not find any differences in the expression of insulin mRNA or in glucose oxidation in cells cultured with the different fatty acids.

For all 4 fatty acids investigated, we found a 2.5- to 4-fold increase in BIS after long-term exposure and a maximum

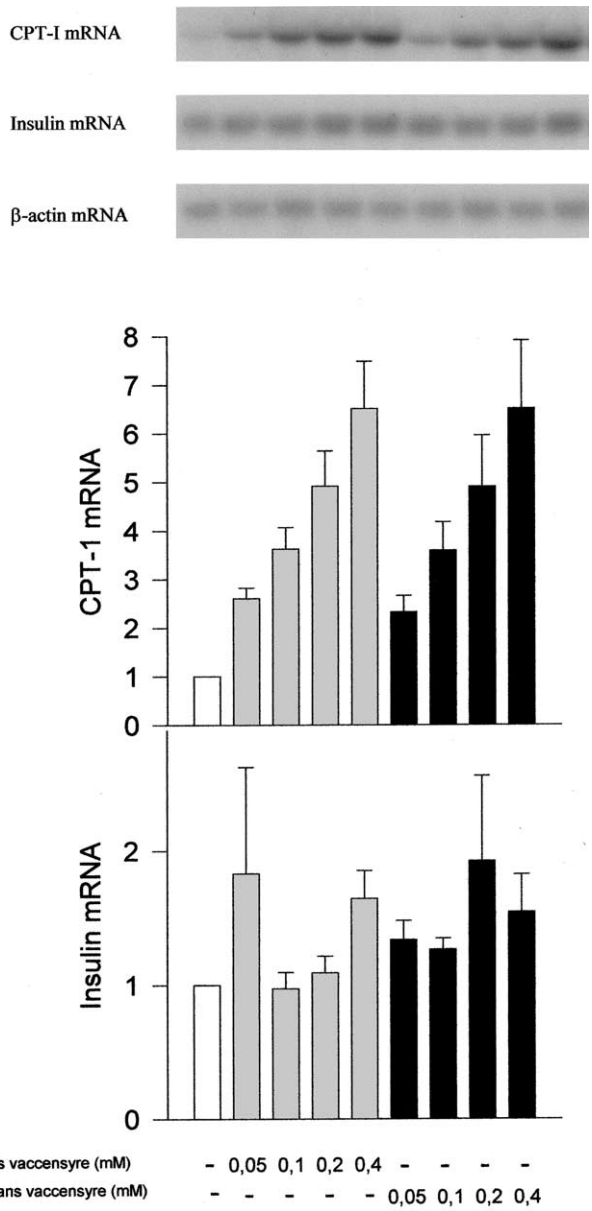

Fig 3. Expression of CPT-I and insulin mRNA after culture with *cis* and *trans* vaccenic acid (0.05 to 0.4 mmol/L) at 5.0 mmol/L glucose for 24 hours. The upper panel shows the CPT-I, insulin, and β-actin mRNA for 1 representative experiment. The lower panel shows the expression of CPT-I mRNA as well as insulin mRNA in INS-1 cells cultured with *cis* vaccenic acid (grey bars) or *trans* vaccenic acid (black bars) (0.05 to 0.4 mmol/L) given relative to control (cells cultured with 0.5% BSA without addition of fatty acids) (white bar). Data are given as the mean ± SEM of 6 different experiments.

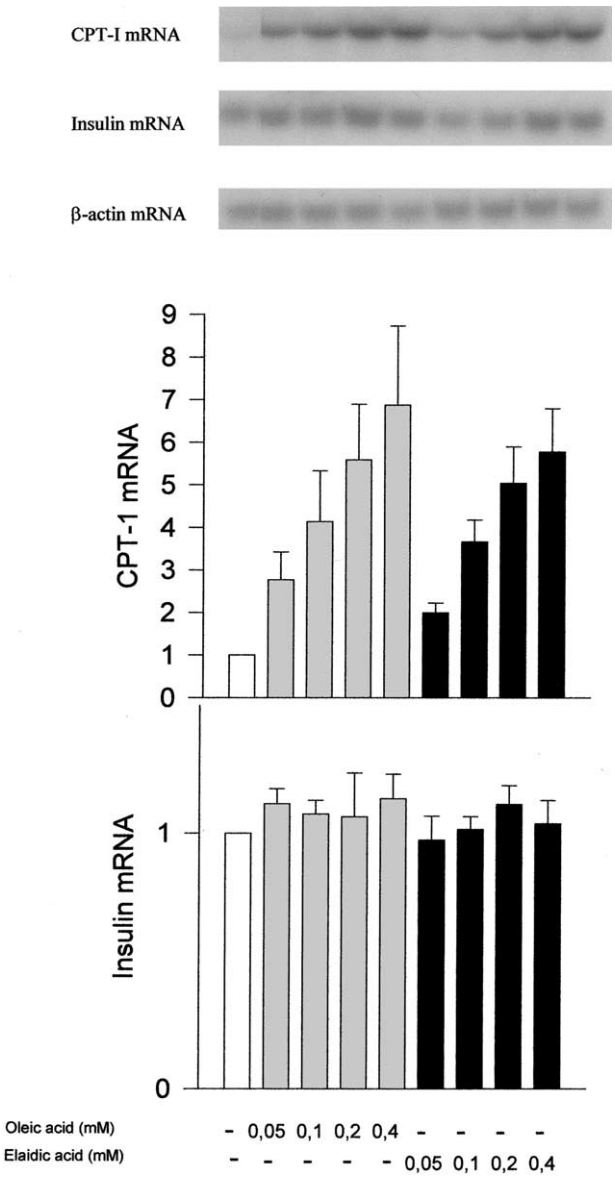

Fig 4. Expression of CPT-I and insulin mRNA after culture with oleic and elaidic acid (0.05 to 0.4 mmol/L) at 5.0 mmol/L glucose for 24 hours. The upper panel shows the CPT-I, insulin, and β-actin mRNA for 1 representative experiment. The lower panel shows the expression of CPT-I mRNA as well as insulin mRNA in INS-1 cells cultured with oleic acid (grey bars) or elaidic acid (black bars) (0.05 to 0.4 mmol/L) given relative to control (cells cultured with 0.5% BSA without addition of fatty acids) (white bar). Data are given as the mean ± SEM of 6 different experiments.

response at 0.4 mmol/L fatty acid, irrespective of the type of fatty acid studied. This corroborates with studies in both islets[5,7] and INS-1 cells[17,18] where long-term exposure to fatty acid increased the basal insulin secretion 2- to 4-fold. Interestingly, *cis* vaccenic acid caused a 40% to 60% higher BIS than *trans* vaccenic acid, a phenomenon that is probably not a common feature for all isomers of fatty acids since the BIS was similar to with oleic and elaidic acid. It might be questioned if culture of INS-1 cells in an even higher fatty acid concentration (>0.4 mmol/L) would equalize the differences in BIS induced by *cis* and *trans* vaccenic acid. However, at concentrations above 0.4 mmol/L both *cis* and *trans* vaccenic acid were toxic, causing cell death.

Addition of *cis* nor *trans* vaccenic acid did not alter the GSIS; however, with increasing fatty acid concentration the incremental response to elevated glucose seems to decline. For 0.3 to 0.4 mmol/L *cis* vaccenic acid and for 0.4 mmol/L *trans* vaccenic acid, there were no significant difference between BIS and GSIS. The same tendency was also found for oleic acid, since the incremental response to high glucose diminished with increasing fatty acid concentration, and at 0.4 mmol/L oleic acid there was no difference between GSIS and BIS. Interestingly, INS-1 cells cultured with elaidic acid retained the ability to further increase insulin secretion to high glucose at all fatty acid concentrations investigated. Thus, the extent of the pathological changes in β-cell function induced by fatty acids was clearly dependent on the type of fatty acid. Both *trans* fatty acids tested (elaidic and *trans* vaccenic acid) seem to have less pathologic effect on β-cell function compared to the corresponding *cis* isomers (oleic and *cis* vaccenic acid, respectively). The potential physiological importance of this is unknown since the long-term effect of isomeric fatty acids on peripheral insulin resistance has not yet been clarified. Surprisingly, the 2 *trans* fatty acids differed in their way of influencing the β-cell functions, ie, *trans* vaccenic acid only had minor effect on BIS compared to *cis* vaccenic acid, while the differences in β-cell response to elaidic acid compared to oleic acid were most pronounced for GSIS. This may indicate that both the spatial form as well as the position of the double bond in unsaturated fatty acids may be of importance for the long-term effect on β-cell function.

Fatty acids are tightly bound to albumin, and it is well known that it is the free, unbound concentration of fatty acids that is of importance for the insulinotropic effect.[2,12] Our experiments have been performed with 0.5% albumin, which is well below the physiological level of 3% to 4 %. Consequently, we operate with total fatty acids concentrations 6 to 8 times lower than the amount that would be relevant at 3% to 4% albumin. The differential effects of *cis* and *trans* fatty acids appear at fatty acid concentrations of 0.2 to 0.4 mmol/L. This would correspond to concentrations of 1.2 to 2.4 mmol/L at 3% to 4% albumin, fatty acid levels observed in type 2 diabetes, which underlines the physiological relevance of the results.

Previously, we have demonstrated that the increased BIS and blunted insulin responsiveness to fatty acids were associated with changes in expression of a multitude of genes.[28,29] The induced expression of genes involved in fatty acid oxidation and the downregulated gene expressions involved in insulin signal transduction and glycolysis[28,29] may contribute to changes in insulin secretion from β cells exposed to fatty acids. Consequently, deterioration in β-cell function caused by chronic exposure to elevated fatty acids has been related to changes in fatty acid and glucose oxidation. Prentki et al[30] proposed that the concentration of long-chain acyl coenzyme A (LC acylCoA) in the cytoplasma determines the amount of secreted insulin. The concentration of LC acylCoA is regulated by the input from the exterior and by the disappearance into the

mitochondria, which is facilitated by the CPT-I complex.[31] The activity of the CPT-I complex is inhibited by malonylCoA, which may be generated when glucose is oxidized.

Reports have shown that chronic exposure of β cells to elevated levels of fatty acids decreases glucose oxidation in both islets[7,9] and β-cell lines.[17,18,32] However, the opposite phenomenon has also been demonstrated, ie, long-term exposure to fatty acids has been associated with increased glucose oxidation at normal (3.3 to 10 mmol/L) glucose concentration.[8,11,33] In this study we could not demonstrate any influence of long-term exposure to fatty acids on glucose oxidation in INS-1 cells, which may indicate that the generation of malonylCoA by a glucose stimulus is equal irrespective of whether the cells are treated with fatty acids or not. This seems to conform with our fatty acid oxidation measurements, where we found an almost equal (37% to 46%) inhibition of fatty acid oxidation when glucose was increased from 3.3 to 16.7 mmol/L, irrespective of culturing conditions.

Chronic exposure of the β cell to elevated levels of fatty acids causes an increased fatty acid delivery to the β cell from the exterior and perhaps from increased intracellular triglyceride (TG) stores,[34] which tend to elevate the cytosolic LC acylCoA content. However, this may in part be counteracted by increased fatty acid oxidation, and a new balance may develop with an increased cytosolic content of LC acylCoA enhancing BIS. Furthermore, increased TG stores in the pancreas have been associated with deteriorated β-cell function[35] and it might be expected that cells displaying a high rate of fatty acid oxidation have minor intracellular TG stores. It is tempting to speculate if there is a connection between fatty acid–induced changes in fatty acid oxidation and the deteriorated insulin secretion. Apparently, cells chronically exposed to 0.4 mmol/L elaidic acid display fewer abnormalities in insulin secretion compared to oleic acid, and we found a relative higher fatty acid oxidation for elaidic acid. In addition, *trans* vaccenic acid, which has a relative high fatty acid oxidation, displayed lower BIS than did *cis* vaccenic acid.

Obviously, it cannot be ruled out that changes in fatty acid oxidation and insulin secretion to different fatty acids simply are independent phenomena and that (an)other mechanism(s) causes each or both.

It should be stressed that we have used a labeled derivate of the most common fatty acid, [U-^{14}C] palmitate, to measure the capacity of fatty acid oxidation which, however, may not be equal to the oxidation of the 4 fatty acids examined in this study. Furthermore, after long-term culture with the isomeric fatty acids, these may be incorporated into TG stores in the β cell and subsequently be released during the performance of the fatty acid oxidation experiment. We cannot exclude that the released LC acylCoA may interfere with the oxidation of [U-^{14}C]palmitate. An estimation of the relevance of these considerations will need further investigation.

A previous study[17] has shown that the increased fatty acid oxidation in fatty acid–treated β cells may be caused by an increase in CPT-I mRNA content and CPT-I activity. Thus, we wanted to clarify whether the differential effects of *cis* and *trans* fatty acids on fatty acid oxidation could be ascribed to differences in the expression of CPT-I mRNA. As expected, all fatty acids investigated caused a marked induction of the CPT-I transcript; however, we found no difference in the ability of the isomeric fatty acids to accumulate CPT-I mRNA. Thus, the differential effect of isomeric fatty acids on fatty acid oxidation seems related to another mechanism. Studies in mitochondria from liver,[36] heart,[37] and skeletal muscle[37] isolated from rats being fed diets containing various amounts and compositions of fat have shown a different influence on CPT-I activity and sensitivity to malonylCoA. It was suggested that the different fatty acid composition of the diets modulates the membrane composition next to the CPT-I and thereby the function of the CPT-I complex. Theoretically, incorporation of *cis* and *trans* fatty acids in the mitochondrial membrane might affect the CPT-I complex and thereby fatty acid oxidation differentially. However, numerous other possibilities exist, eg, the translation of the CPT-I mRNA or the β-oxidation in the mitochondria could be differentially affected by isomeric fatty acids.

Contrasting data exist on the effect of fatty acids on insulin mRNA expression, with some studies reporting a decrease[21,22] and others an increase.[20] In our experiments we did not find any changes in the insulin mRNA content irrespective of whether cells were cultured with fatty acids or not, indicating that fatty acids do not influence the transcription of the insulin gene. However, any effect on the translation of insulin mRNA cannot be excluded.

In summary, we have demonstrated that long-term exposure of INS-1 cells to isomeric fatty acids causes a differential response in the secretory capacity of the β cell with the fatty acid –induced changes being most pronounced for *cis* fatty acids compared to *trans* fatty acids. The mechanism behind this differential effect may be related to a relative high increase in fatty acid oxidation in cells treated with *trans* isomers compared to *cis* isomers. The differences in fatty acid oxidation seem not to be ascribed to differences in the potency of induction CPT-1 mRNA. Neither did the glucose oxidation nor the insulin mRNA content show any differences in response to culture with isomeric fatty acids.

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REFERENCES

1. Sako A, Grill V: A 48-hour lipid infusion in the rat time-dependently inhibits glucose-induced insulin secretion and B-cell oxidation through a process likely coupled to fatty acid oxidation. J Endocrinol 127:1580-1589, 1990

2. 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

3. Conget I, Rasschaert J, Sener A, et al: Secretory, biosynthetic,

respiratory, cationic, and metabolic responses of pancreatic islets to palmitate and oleate. Biochem Med Metabol Biol 51:175-184, 1994

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

5. 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

6. Zhou YP, Grill V: Long term exposure to fatty acids and ketones inhibits beta-cell functions in human pancreatic islets of Langerhans. J Clin Endocrinol Metab 80:1584-1590, 1995

7. 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

8. 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

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

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

11. 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

12. 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

13. 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

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. Alstrup KK, Gregersen S, Jensen HM, et al: Differential effects of cis and trans fatty acids on insulin release from isolated mouse islets. Metabolism 48:22-29, 1998

16. Haber EP, Xiemenes HMA, Procopio J, et al: Pleiotropic effects of fatty acids on pancreatic β-cells. J Cell Physiol 194:1-12, 2002

17. Assimacopoulos-Jeannet F, Thumelin S, Roche E, et al: Fatty Acids rapidly induce the carnitine palmitoyltransferase I gene in the pancreatic beta-cell line INS-1. J Biol Chem 272:1659-1664, 1997

18. Brun T, Assimacopoulos-Jeannet F, Corkey BE, et al: Long-chain fatty acids inhibit acetyl-CoA carboxylase gene expression in the pancreatic beta-cell line INS-1. Diabetes 46:393-400, 1997

19. Zhou YP, Priestman DA, Randle PJ, et al: Fasting and decreased B cell sensitivity: Important role for fatty acid-induced inhibition of PDH activity. Am J Physiol 270:E988-E994, 1996

20. Bollheimer LC, Skelly RH, Chester MW, et al: Chronic exposure to free fatty acid reduces pancreatic β cell insulin content by increasing basal insulin secretion that is not compensated for by a corresponding increase in proinsulin biosynthesis translation. J Clin Invest 101:1094-1101, 1998

21. Capito K, Hansen SE, Hedeskov CJ, et al: Fat-induced changes in mouse pancreatic islet insulin secretion, insulin biosynthesis and glucose metabolism. Diabetol Acta 28:193-198, 1992

22. Gremlich S, Bonny C, Waeber G, et al: Fatty acids decrease IDX-1 expression in rat pancreatic islets and reduce GLUT2, glucokinase, insulin and somatostatin levels. J Biol Chem 272:30261-30269, 1997

23. Asfari M, Janjic D, Meda P, et al: Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. Endocrinology 130:167-178, 1992

24. Bradford MM: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254, 1976

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

26. Gliemann J: Insulin-like activity of dilute human serum assayed by an isolated adipose cell method. Diabetes 14:643-646, 1965

27. Sambrook J, Fritsch EF, Maniatis T, et al: Molecular Cloning. A Laboratory Manual (ed 2). New York, NY, Cold Spring Harbor Laboratory Press, 1989

28. Xiao J, Gregersen S, Pedersen SB, et al: Differential impact of acute and chronic lipotoxicity on gene expression in INS-1 cells. Metabolism 51:155-162, 2002

29. Xiao J, Gregersen S, Pedersen SB, et al: The effect of chronic exposure to fatty acid on gene expression in clonal insulin-producing cells: Studies using high density oligonucleotide microarray. Endocrinology 142:4777-4784, 2001

30. Prentki M, Corkey BE: Perspectives in diabetes. Are the beta-cell signaling molecules malonyl-CoA and cytosolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM. Diabetes 45:273-283, 1996

31. McGarry JD, Brown NF: The mitochondrial carnitine palmitoyltransferase system—From concept to molecular analysis. Eur J Biochem 244:1-14, 1997

32. Liang Y, Buettger C, Berner DK, et al: Chronic effect of fatty acids on insulin release is not through the alteration of glucose metabolism in a pancreatic beta-cell line (betaHC9). Diabetologia 40:1018-1027, 1997

33. Hosokawa H, Corkey BE, Leahy JL: Beta-Cell hypersensitivity to glucose following 24-h exposure of rat islets to fatty acids. Diabetologia 40:392-397, 1997

34. Lee Y, Hirose H, Zhou Y, et al: Increased lipogenic capacity of the islets of obese rats. Diabetes 46:408-413, 1997

35. Koyama K, Chen G, Lee Y, et al: Tissue triglyceride, insulin resistance, and insulin production: implications for hyperinsulinemia of obesity. Am J Physiol 273:E708-E713, 1997

36. Power GW, Yaqoob P, Harvey DJ, et al: The effect of dietary lipid manipulation on heptic mitochondrial phospholipid fatty acid composition and carnitine palmitoyltransferase I activity. Biochem Mol Biol Int 34:671-684, 1994

37. Power GW, Newsholme EA: Dietary fatty acids influence the activity and metabolic control of mitochondrial carnitine palmitoyltransferase I in rat heart and skeletal muscle. J Nutr 127:2142-2150, 1997