Rapid Publication

Streptozotocin Treatment Upregulates Uncoupling Protein 3 Expression in the Rat Heart

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Diabetic rats have a deficiency in their heart ATP concentrations, and although the mechanism remains to be elucidated, this deficiency may involve increased uncoupling of oxidative phosphorylation. To investigate whether heart uncoupling proteins (UCPs) are subject to transcriptional regulation in diabetes, we examined changes in UCP mRNA expression in the heart of streptozotocin-induced diabetic (STZ-DM) rats. Heart UCP3 mRNA expression significantly increased by 9.4-fold in STZ-DM rats, while levels of UCP2 mRNA expression were not significantly altered. Insulin supplementation in STZ-DM rats returned UCP3 mRNA concentrations to control levels. The expression of UCP3 mRNA was similarly elevated in the heart of fasted rats, which also have hypoinsulinemia and hyper–free fatty acidemia but, unlike the STZ-DM rats, are hypoglycemic. Since hyperinsulinemia alone was previously reported to not affect UCP3 gene expression in the muscle, these results indicate that hyper–free fatty acidemia is a potent enhancer of UCP3 gene expression in the diabetic rat heart. Interestingly, we found no changes in UCP3 mRNA levels in Zucker fatty (*falfa*) rats with excessive chronic hyper–free fatty acidemia, which suggests that upregulation of heart UCP3 mRNA may depend on an acute change in free fatty acid concentrations rather than on their sustained elevation. High-energy ATP deficiencies in the diabetic rat heart may primarily result from proton leakage due to the upregulation of UCP3 expression.
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Uncoupling proteins (UCPs), which are inner mitochondrial proton transporters, stimulate heat production by uncoupling respiration from ATP synthesis (1,2). The third member of the UCP family, UCP3, is expressed excessively in skeletal muscle and in brown adipose tissue, both of which are thermogenic tissues in which energy expenditure is regulated in mammals (3–5). UCP3 mRNA is also expressed at low levels in heart (3–5), though its potential function in that tissue has not yet been studied. Moreover, while its thermogenic regulation in brown adipose tissue and skeletal muscle is well understood, its presence and potential role in heart, where increased heat production could be detrimental, remains to be elucidated.

While cardiovascular complications are commonly associated with diabetes, a unique type of cardiomyopathy has been described in diabetic patients that is independent of atherosclerosis of the coronary artery, hypertension, and valvular diseases (6–8). In fact, impaired ventricular performance resulting in decreased cardiac output in diabetic animals was shown to be attributable to abnormalities in myocardial metabolism (9,10). The heart in a normal steady state utilizes fatty acids and glucose at a ratio of 7:3 for cardiac ATP production (11). In the diabetic rat heart, however, concomitant with an elevation in circulating free fatty acids (FFAs) (12), FFAs are used as the primary (99%) source of myocardial oxidative fuel, to the almost complete exclusion of glucose. In spite of the accelerated FFA β-oxidation described, intracellular ATP levels were actually decreased, rather than increased, in the diabetic heart (13–17). Along with the activation of both adenosine deaminase and AMP deaminase (14), this reduction in ATP levels may result from either an inactivation of oxidative phosphorylation due to a shortage in oxygen delivery (13) and/or an increase in the uncoupling process, the mechanism for which has not yet been characterized.

Recently, FFAs were reported to upregulate UCP3 gene expression in rat skeletal muscle (18). Moreover, FFAs have been found to play an important role as signaling molecules in triggering a signal transduction pathway (19) and as activators of peroxisome proliferator-activated receptor (PPAR) (20). These findings raise the question of whether heart UCPs are similarly enhanced in diabetic animals, since circulating FFAs are elevated in these animals. The present study investigates potential changes in heart UCP2 and UCP3 mRNA levels in streptozotocin (STZ)-induced diabetic (STZ-DM) rats and assesses whether FFAs may modulate UCP expression, using

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CIT-CON, citrate buffer control; CIT-SAL, CIT-CON rats with saline treatment; EtBr, ethidium bromide; FFA, free fatty acid; H-FABP, heart-type fatty-acid–binding protein; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator-response element; RIA, radioimmunoassay; STZ, streptozotocin; STZ-DM, STZ-induced diabetic; STZ-INS, STZ-DM rats with insulin treatment; STZ-SAL, STZ-DM rats with saline treatment; T_3, triiodothyronine; UCP, uncoupling protein.

both fasting and Zucker fatty (*fa*/*fa*) rats that have excessive acute or chronic hyper–free fatty acidemia, respectively. Based on our present results, we propose that upregulation of heart UCP3 gene expression is mediated by increased FFA circulation, resulting in reduced cellular ATP levels at acute stages of diabetes, but not at later stages of the disease.

RESEARCH DESIGN AND METHODS

Animals. Male Wistar King A (WKA) rats, 13–16 weeks of age, and male Zucker fatty (*fa*/*fa*) rats and their lean littermates (*fa*/+), 18–19 weeks of age, were used in these studies. The genotype of the Zucker rats was confirmed using the method of Phillips et al. (21). All rats were housed in a room illuminated daily from 0700 to 1900 (12:12 h light/dark cycle) with temperature maintained at 21 ± 1°C and humidity at 55 ± 5%. All animals, unless otherwise described, were allowed free access to tap water and standard pellet rat diet (CE-2; Clea Japan, Tokyo).

Preparation of acute-phase STZ-DM rats. A solution of STZ (Sigma, St. Louis, MO) was freshly prepared on day 0 of the experiment by dissolving it to a final concentration of 0.1 mol/l (pH 4.5) in sterile sodium citrate buffer. There were 10 male WKA rats assigned to one of two groups, the STZ-DM group (n = 5) or the citrate buffer control (CIT-CON) group (n = 5), matched by body weight and age. Rats were made diabetic by injecting 0.6 ml STZ (60 mg/kg) into the tail vein at 1900–1910 under light ether anesthesia. The CIT-CON group was injected with the same volume of citrate buffer solution containing no STZ. The rats in the STZ-DM group were considered diabetic when their blood glucose concentrations, measured from an intravenous catheter (see below), were >350 mg/dl in the fed state 2.5 days after the STZ injection.

Preparation of later-phase and insulin-supplemented STZ-DM rats. There were 12 male WKA rats assigned to one of three groups: STZ-DM rats with insulin treatment (STZ-INS) (n = 4), STZ-DM rats with saline treatment (STZ-SAL) (n = 4), or CIT-CON rats with saline treatment (CIT-SAL) (n = 4). After STZ treatment proceeded as described above, the STZ-INS group was treated with subcutaneous injections of 3 U insulin/day (Lente insulin; Shimizu, Shizuoka, Japan) at 1900–1910 for 6 days, starting on day 15 and continuing to day 20 after the STZ injection. The STZ-SAL and the CIT-SAL groups were similarly injected with an equal volume of saline.

Food deprivation procedure. Matched on the basis of body weight and age, 10 WKA rats were assigned to either the ad libitum–fed control group or the fasted group (n = 5 per group). Food was withdrawn for 48 h, starting at 0700. Rats had free access to tap water during the fasting period.

Blood sampling and heart extirpation. Blood samples were collected from each rat through a chronically indwelling silicone catheter implanted in the right external jugular vein with its end at a point just outside of the atrium (22). Surgical catheterizations were done under ether anesthesia 7 days before the STZ injections. Just before removal of the heart of each rat, blood samples were taken at 0700–0710. Serum samples were immediately frozen at –20°C until humoral factor levels were measured. Hearts, surgically removed at 0710–0720 under ether anesthesia, were immediately frozen in liquid nitrogen. All tissue samples were stored at –80°C until RNA extraction.

Assays of blood- and adipocyte-born humoral factors. Serum insulin and leptin levels were quantitated using an insulin radioimmunoassay (RIA) kit (Rat insulin [^{125}I] assay system; Amersham, Little Chalfont, U.K.) and a rat leptin RIA kit (Linco, St. Louis, MO), respectively. Serum glucose, total cholesterol, triglyceride, and FFA concentrations were measured with commercially available kits (Merckauto Glucose; Kanto Chemical, Tokyo; L Type Wako Cholesterol and L Type Wako Triglyceride; Wako Pure Chemical, Osaka, Japan; NEFA-SS'Eiken; Eiken Chemical, Tokyo).

RNA extraction and Northern blot analysis. Total RNA was extracted using the standard acid guanidinium phenolchloroform method (23). Northern blot analysis was performed as described previously (24). cDNA probes for the rat glucose transporter 4 (GLUT4), heart-type fatty-acid–binding protein (H-FABP), UCP2, and UCP3 were prepared by reverse transcription–polymerase chain reaction using the following primers: GLUT4 sense, 5′-TCT GGC TAT CAC AGT ACT CC-3′; GLUT4 antisense, 5′-TCT GTA CTG GGT TTC ACC TC-3′; H-FABP sense, 5′-CTC ACG ACC GAC ATC GAC CT-3′; H-FABP antisense, 5′-GGT GGC CTT GGC TCT GCT TT-3′; UCP2 sense, 5′-CAT CTT CTG GGA GGT AGC-3′; UCP2 antisense, 5′-AAG ACA GGG CAG GAA TGG-3′; UCP3 sense, 5′-GTT ACC TTT CCA CTG GAC AC-3′; and UCP3 antisense, 5′-CCG TTT CAG CTG CTC ATA GG-3′. The identity of the appropriately sized product was confirmed by mapping with multiple restriction endonucleases and sequencing. The hybridization signals were analyzed with a BIO-image analyzer BAS 2000 (Fuji, Tokyo). The density of 28S ribosomal RNA (rRNA), stained with ethidium bromide (EtBr) and detected with ultraviolet transillumination, was used to monitor the amount of total RNA in each sample (25,26).

Statistical analysis. Data are expressed as means ± SE. Statistical significances between two groups were assessed by unpaired Student's t test and between three groups by one-way analysis of variance followed by Scheffé's multiple comparison test. Probability of 0.05 was used as the threshold for statistical significance.

RESULTS

Metabolic changes and utilization of fatty acids in heart from acute-phase STZ-DM rats. The STZ-DM group showed weight loss (P < 0.01), hyperglycemia (P < 0.0001), and hypoinsulinemia (P < 0.0001) 2.5 days after STZ treatment (Table 1). In the STZ-DM group, serum FFA levels were increased by more than threefold (P < 0.0001), accompanied by an increase in serum triglyceride levels of more than fourfold (P < 0.01) and a decrease in serum leptin levels of 2.8-fold (P < 0.0001). Serum cholesterol concentrations were not affected by STZ treatment. To determine whether fatty acids were the preferred energy source in the heart of the STZ-DM rat, changes in GLUT4 and H-FABP gene expression were measured. As shown in Fig. 1*A* and *B*, in the STZ-DM heart, GLUT4 and H-FABP mRNAs were reduced by more than one-third and increased by more than twofold, respectively, compared with those in the CIT-CON group (P < 0.0001 for each).

Changes in heart UCP gene expression in acute-phase STZ-DM rats. A dramatic 9.4-fold augmentation in the expression of UCP3 mRNA was induced in the STZ-DM group compared with that in the CIT-CON group (P < 0.0001) (Fig. 1*A* and *B*). Extended electrophoretic analysis revealed a separation of two UCP3 forms and indicated that the relative ratio of the major ($UCP3_S$) and minor ($UCP3_L$) bands was not significantly changed between the two groups (Fig. 1*C*). In contrast to the marked increase in UCP3 mRNA, UCP2 mRNA was not significantly changed in the STZ-DM heart compared with that in the CIT-CON heart (Fig. 1*A* and *B*).

Changes in heart UCP gene expression and levels of humoral factors in later-phase STZ-DM rats after insulin supplementation. The effects of prolonged diabetic condition and of insulin treatment in later-phase STZ-DM rats on heart UCP mRNA expression and blood- and adipocyte-born humoral factors are summarized in Fig. 2 and Table 2. After chronic diabetes, the levels of serum metabolites were similar to those seen in the acute STZ-DM group shown in Table 1, with the exception of total cholesterol levels, which were increased in later-phase STZ-SAL rats (CIT-SAL vs. STZ-SAL, P < 0.05). Insulin supplementation in the STZ-DM rats returned FFAs and leptin levels, as well as insulin levels, to those observed in the CIT-SAL group (FFA, leptin, and insulin; no significant difference between CIT-SAL and STZ-INS). Insulin treatment also

TABLE 1
Changes in body weight and serum metabolite in response to STZ treatment

	CIT-CON	STZ-DM
Body weight (g)	306 ± 6	276 ± 5*
Glucose (mg/dl)	126 ± 2	472 ± 20†
Insulin (ng/ml)	10.9 ± 1.0	2.4 ± 0.1†
Total cholesterol (mg/dl)	62 ± 2	68 ± 3
Triglyceride (mg/dl)	83 ± 6	398 ± 63*
FFAs (mEq/l)	0.33 ± 0.02	1.13 ± 0.07†
Leptin (ng/ml)	2.8 ± 0.2	1.0 ± 0.1†

Data are means ± SE; n = 5 per group. *P < 0.01, †P < 0.0001 compared with the CIT-CON group.

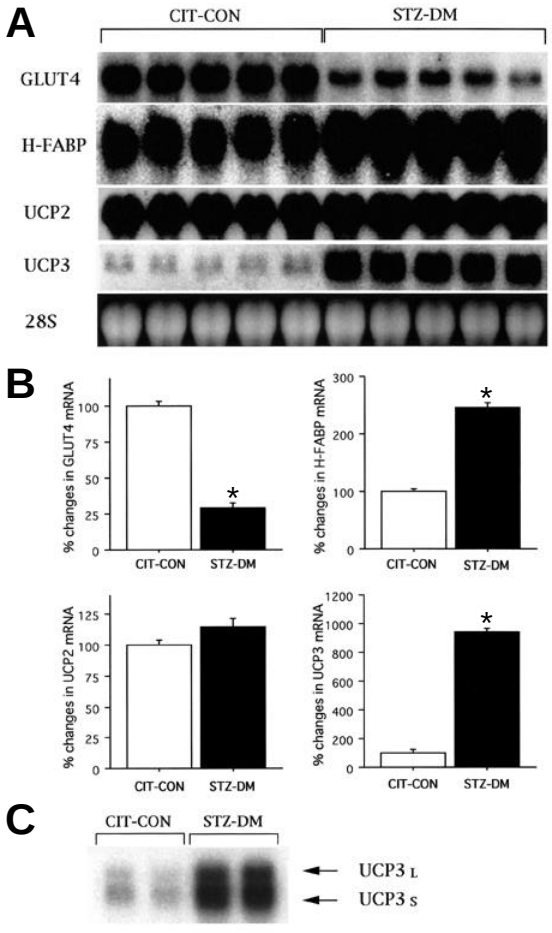

FIG. 1. Regulation of mRNA expression of GLUT4, H-FABP, UCP2, and UCP3 in the hearts of STZ-DM rats. *A*: Northern blots and corresponding EtBr-stained 28S rRNA electrophoretically separated on an agarose gel. Total RNA (20 µg/lane) was analyzed with each lane containing RNA from one rat. *B*: Quantitation of the percent changes in GLUT4, H-FABP, UCP2, and UCP3 mRNA levels in the STZ-DM heart from those in the CIT-CON heart. Data are means ± SE (n = 5 per group). *P < 0.0001 compared with the corresponding CIT-CON group. *C*: Representative blots from an extended electrophoretic UCP3 Northern blot analysis. The ratios of $UCP3_S$, a major band, and $UCP3_L$, a minor band, did not change between the groups.

restored concentrations of serum glucose and triglycerides (STZ-SAL vs. STZ-INS: P < 0.01 for each), but not to the levels observed in the CIT-SAL group (CIT-SAL vs. STZ-INS: glucose, P < 0.01; triglycerides, P < 0.05) (Table 2). As was shown by acute STZ treatment (Fig. 1), heart UCP3 mRNA expression was increased in later-phase STZ-SAL rats (4.2-fold, P < 0.001), while heart UCP2 mRNA expression was not affected (Fig. 2). This augmenting effect of STZ on UCP3 expression was completely abolished by insulin replacement, which returned UCP3 mRNA levels to those in CIT-SAL animals. No changes in UCP2 mRNA levels were observed in the STZ-INS group (Fig. 2).

Changes in heart UCP gene expression after fasting. Metabolic changes in response to fasting are shown in Table 3. Our data clearly show that 48 h of fasting resulted in hypoinsulinemia (P < 0.0001), hypoglycemia (P < 0.0001), and hypotriglyceridemia (P < 0.01), which were concomitant with hyper–free fatty acidemia (P < 0.0001) and hypoleptinemia (P < 0.0001). Heart UCP3 mRNA expression

TABLE 2
Effects of insulin supplementation on body weight and serum metabolite in STZ-induced diabetic rats

	CIT-SAL	STZ-SAL	STZ-INS
Body weight (g)	353 ± 4	259 ± 5*	307 ± 7†‡
Glucose (mg/dl)	95 ± 2	524 ± 8*	295 ± 59†§
Insulin (ng/ml)	7.7 ± 1.1	2.1 ± 0.2‖	8.5 ± 1.4§
Total cholesterol (mg/dl)	65 ± 1	84 ± 4‖	81 ± 1‖
Triglyceride (mg/dl)	59 ± 4	254 ± 21*	40 ± 9§‖
FFAs (mEq/l)	0.39 ± 0.02	0.74 ± 0.02*	0.34 ± 0.02‡
Leptin (ng/ml)	2.4 ± 0.2	0.7 ± 0.4¶	2.0 ± 0.2§

Data are means ± SE; n = 4 per group. *P < 0.0001, †P < 0.001 compared with the CIT-SAL group. ‡P < 0.0001, §P < 0.01 compared with the STZ-SAL group. ‖P < 0.05, ¶P < 0.01 compared with the CIT-SAL group.

increased by 1.6-fold in 48-h fasted rats (P < 0.0001), compared with that in the ad libitum–fed control group (Fig. 3). Unlike UCP3, UCP2 mRNA in the heart decreased slightly but significantly after 48 h of fasting (P < 0.01) (Fig. 3).

Changes in heart UCP gene expression in Zucker rats. Zucker fatty (*fa/fa*) rats were markedly obese with hyperlipidemia. Serum FFA concentrations were elevated in Zucker fatty rats on average to 0.58 ± 0.04 mEq/l, compared with those in the lean littermates (0.33 ± 0.06 mEq/l) (P < 0.01). In contrast, heart UCP2 and UCP3 mRNA levels were not altered in either Zucker fatty rats or in their lean littermates (Fig. 4).

DISCUSSION

In the present study, expression of heart UCP3 mRNA was increased in the STZ-DM group, and its expression remained elevated throughout the 3-week testing period. The expression of UCP2 mRNA, however, was not altered. The increase in UCP3 expression was shown for both the $UCP3_L$ and $UCP3_S$

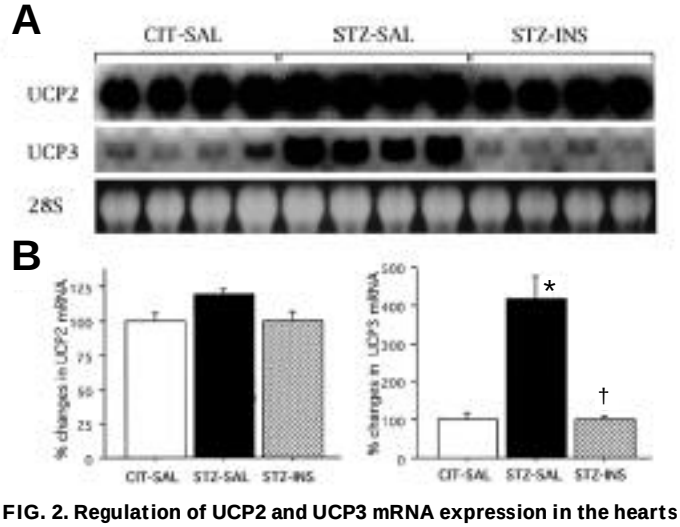

FIG. 2. Regulation of UCP2 and UCP3 mRNA expression in the hearts of STZ-DM and STZ-INS rats. *A*: Northern blot and corresponding EtBr-stained 28S rRNA electrophoretically separated on an agarose gel. Total RNA (20 µg/lane) was analyzed with each lane containing RNA from one rat. *B*: Quantitation of the percent changes in UCP2 and UCP3 mRNA levels in heart from STZ-SAL and STZ-INS rats from that in CIT-SAL rats. Data are means ± SE (n = 4 per group). *P < 0.001 compared with the corresponding CIT-SAL group; †P < 0.001 compared with the corresponding STZ-SAL group.

TABLE 3
Changes in body weight and serum metabolite in response to fasting

	Ad libitum–fed	Fasted
Body weight (g)	347 ± 4	304 ± 6*
Glucose (mg/dl)	121 ± 2	85 ± 2†
Insulin (ng/ml)	6.8 ± 0.3	3.4 ± 0.3†
Total cholesterol (mg/dl)	63 ± 2	75 ± 3*
Triglyceride (mg/dl)	82 ± 5	32 ± 3*
FFAs (mEq/l)	0.41 ± 0.03	0.86 ± 0.01†
Leptin (ng/ml)	2.1 ± 0.1	0.9 ± 0.1†

Data are means ± SE; n = 5 per group. *P < 0.01, †P < 0.0001 compared with the ad libitum–fed group.

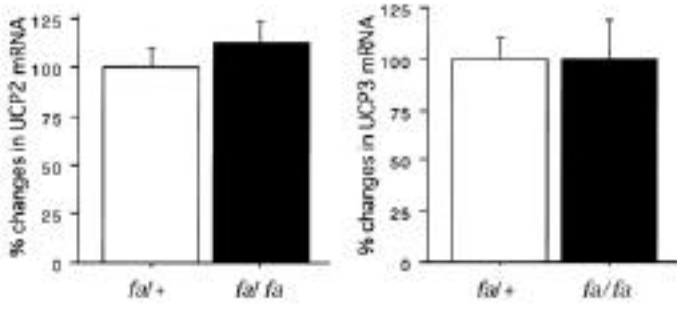

FIG. 4. Northern blot analysis of heart UCP2 and UCP3 mRNA from 18- to 19-week-old male Zucker fatty (*falfa*) rats and their lean littermates (*fal*+). Percent changes in heart UCP2 and UCP3 mRNA levels in the Zucker fatty (*falfa*) rats from those in lean littermates (*fal*+) are depicted. Data are means ± SE (n = 5 per group).

forms. $UCP3_L$ possesses an uncoupling activity similar to that of UCP1 and UCP2 (27,28). On the other hand, $UCP3_S$ lacks the purine nucleotide binding domain in the COOH-terminal region of the protein that is responsible for the downregulation of UCP1 uncoupling activity (29,30). Thus, $UCP3_S$ may have even stronger uncoupling properties, though this has not yet been shown. Alternatively, $UCP3_S$ may have no uncoupling activity, specifically due to its deletion in the sixth transmembrane region (3,30). Nevertheless, our present findings support an overall increase in UCP3 uncoupling activity in the diabetic heart, as is suggested by the increase in the expression of both forms of UCP3 in the heart of STZ-DM rats. UCP3 mRNA levels in the heart were restored to control levels after insulin replacement, making it more likely that this increase in expression occurred in response to metabolic changes and not as a nonspecific toxic effect of STZ.

In the present study, there was a clear difference between the regulation of the mRNAs encoding UCP3 and UCP2 in response to STZ treatment. To date, there has been no evidence to suggest or explain such a difference between the regulation of these two UCPs. One possible explanation might attribute this difference to the wide range in their basal expression in the normal rat heart, where UCP3 mRNA is only slightly detectable (3–5) while UCP2 is profoundly expressed (24). These differences in basal expression were also observed in the present study. This would suggest that in the heart, the promoter of the UCP2 gene is already so active under basal conditions that any further induction by other factors remains largely undetectable, yet the low levels of basal UCP3 expression allow it to be more readily and noticeably potentiated in the physiologically challenged heart.

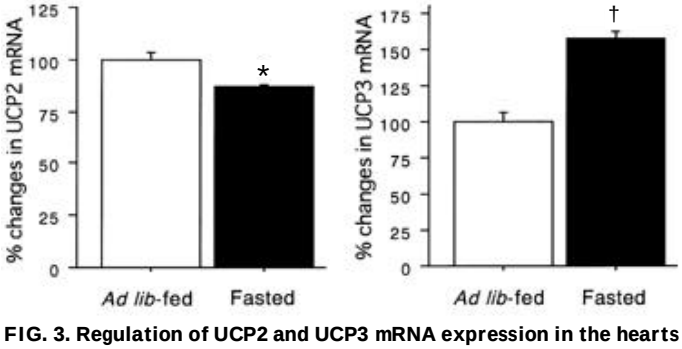

FIG. 3. Regulation of UCP2 and UCP3 mRNA expression in the hearts of fasted rats. Quantitation of the percent changes in UCP2 and UCP3 mRNA levels in the fasted heart (Fasted) from those in the ad libitum–fed heart (*Ad lib*-fed). Data are means ± SE (n = 5 per group). *P < 0.01, †P < 0.0001 compared with the ad libitum–fed group.

Here, we raise the essential question of what the mechanism underlying the upregulation of UCP3 in the diabetic rat heart is. It is possible that insulin deficiency increases heart UCP3 mRNA levels, since insulin replacement restored expression of heart UCP3 mRNA to levels comparable to the CIT-SAL group. Whether insulin deficiency per se or its resulting metabolic abnormalities directly stimulate UCP3 expression is unclear.

Diabetes produces many metabolic abnormalities, including numerous hormonal changes resulting from diminished insulin secretion and/or action. Leptin, a product of the *ob* gene, and triiodothyronine (T_3) have been shown to positively regulate UCP3 gene expression in muscle and adipose tissue (27,31). In the present study, however, serum leptin concentrations were diminished after STZ treatment. Indeed, *ob* mRNA levels were previously reported to be reduced in STZ-DM rats (32), and serum T_3 concentrations were similarly reduced in those rats (33). These findings indicate that leptin and T_3 are not likely to be involved in the upregulation of UCP3 mRNA expression in the diabetic heart.

To further investigate the potential mechanism underlying the increased expression of the UCP3 gene in diabetic hearts, we assessed its expression in the hearts of fasted rats that, like STZ-DM rats, have hypoinsulinemia and hyper–free fatty acidemia, but unlike STZ-treated rats, are hypoglycemic. Both STZ-DM and fasted rats showed an increase in UCP3 mRNA levels, revealing that UCP3 upregulation is independent of serum glucose levels. Moreover, in a euglycemic-hyperinsulinemic clamp study, changes in insulin levels alone did not alter either UCP3 or UCP2 gene expression in humans (34). According to our unpublished data, induction of a hyperinsulinemic hypoglycemic state did not alter UCP3 or UCP2 gene expression in the rat heart. Together, these findings indicate that diabetes-induced UCP3 upregulation in the heart is not dependent on circulating glucose or insulin levels. Alternatively, diabetes and fasting both accelerate lipolysis in adipose tissue, which leads to elevations in circulating FFAs (35). In the present study, GLUT4 mRNA expression was reduced in the heart of STZ-treated rats, while H-FABP expression was enhanced, an effect that is likely due to increased utilization of fatty acids over glucose as an intracellular energy source. Furthermore, in our present STZ-DM

and fasted rats, an elevation in both serum FFA levels and heart UCP3 mRNA expression was observed that could be reversed after diminished hyper–free fatty acidemia (i.e., refeeding; data not shown), as well as after insulin supplementation. In fact, FFAs have been shown to upregulate UCP3 gene expression in muscle (18), a finding that implies that FFAs may also upregulate UCP3 gene expression in the diabetic heart. Fatty acids are now known to play important roles both as energy substrates and as important signaling molecules, including a role in the activation of PPAR (19). Fatty acids are well known to activate expression of a group of genes that contains the peroxisome proliferator-response element (PPRE) (36,37). The PPRE has been identified in the promoter region of the UCP1 gene (38). Moreover, the expression of UCP2 mRNA is induced by thiazolidinedione, a potent affinity ligand for PPAR-γ (39–41). Though its potential presence in the UCP3 gene promoter remains to be investigated, the PPRE, and particularly PPAR-α, which is predominant in heart, may provide one route by which FFAs could directly stimulate UCP3 gene transcription (36,37).

To further investigate the regulation of heart UCP3 gene expression during acute (9.4-fold increase) and chronic (4.2-fold increase) stages of diabetes, its expression was characterized in Zucker fatty (*fa/fa*) rats that have chronic hyper–free fatty acidemia (42,43). In contrast to the upregulated UCP3 gene expression in the STZ-DM heart, there was no difference in heart UCP3 expression between Zucker fatty rats and their lean littermates, even though Zucker fatty rats had significant hyper–free fatty acidemia compared with their lean littermates, which had normal concentrations of circulating FFAs. When Zucker fatty rats are fasted, however, both heart UCP3 gene expression and circulating FFAs are significantly upregulated (S.H., S. Kondou, H.Y., T.S., unpublished observations). Though further studies are clearly needed to clarify the involvement of FFAs in UCP3 expression in the heart, an acute change in FFA concentrations rather than their sustained elevation must be in part responsible for the elevation of UCP3 mRNA.

The reduction in high-energy ATP levels typically seen in the diabetic heart (13–17) has been attributed to a shortage in oxygen delivery (13), as well as an overactivation of adenosine and AMP deaminases (14). The regulatory processes underlying this mechanism, however, have not yet been elucidated. In the present study, we demonstrated in the STZ-DM rat heart that UCP3 gene expression is predominantly enhanced. The resulting excessive uncoupling of oxidative phosphorylation provides further insight into the cause of ATP deficiency in the diabetic heart.

In summary, we have proposed that UCP3 gene expression may be regulated at least in part by FFAs, not only in skeletal muscle, but also in cardiac muscle. These changes of UCP3 mRNA expression in the heart may be involved in the pathogenesis of the metabolic disorders that accompany diabetes.

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Author Queries (please see Q in margin and underlined text)

Q1: “this deficiency” as meant?

Q2: Is “iszilin” correct here?

Q3: “Amersham” meant here instead of “Amarsham”?
Q4: OK to add “a role in the”? If not, please reword for clarity.
Q5: Please provide names of all researchers involved in these unpublished observations.