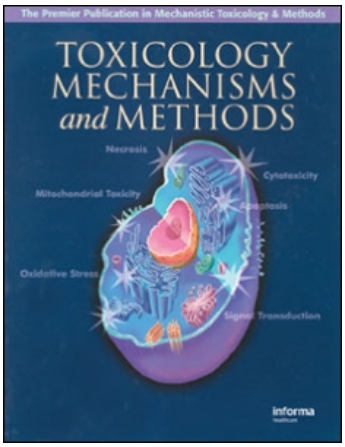

Toxicology Mechanisms and Methods

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713396575

Mitochondrial Bioenergetics, Diabetes, and Aging: Top-Down Analysis Using the Diabetic Goto-Kakizaki (GK) Rat as a Model

Fernanda M. Ferreira [a]; António J. Moreno [a]; Raquel Seiça [b]; Maria S. Santos [a]; Carlos M. Palmeira [a]
[a] Department of Zoology, Center for Neurosciences and Cell Biology of Coimbra, Portugal [b] Faculty of Medicine, University of Coimbra, Portugal

Online Publication Date: 01 August 2006

To cite this Article Ferreira, Fernanda M., Moreno, António J., Seiça, Raquel, Santos, Maria S. and Palmeira, Carlos M.(2006)'Mitochondrial Bioenergetics, Diabetes, and Aging: Top-Down Analysis Using the Diabetic Goto-Kakizaki (GK) Rat as a Model',Toxicology Mechanisms and Methods,16:6,323 — 330

To link to this Article: DOI: 10.1080/15376520600616925

URL: http://dx.doi.org/10.1080/15376520600616925

Toxicology Mechanisms and Methods, 16:323–330, 2006

ISSN: 1537-6516 print / 1537-6524 online
DOI: 10.1080/15376520600616925

Taylor & Francis
Taylor & Francis Group

Mitochondrial Bioenergetics, Diabetes, and Aging: Top-Down Analysis Using the Diabetic Goto-Kakizaki (GK) Rat as a Model

Fernanda M. Ferreira and António J. Moreno
Center for Neurosciences and Cell Biology of Coimbra, Department of Zoology, Portugal

Raquel Seiça
Faculty of Medicine, University of Coimbra, Portugal

Maria S. Santos and Carlos M. Palmeira
Center for Neurosciences and Cell Biology of Coimbra, Department of Zoology, Portugal

In the present study we investigated the changes in the oxidative phosphorylation system of liver mitochondria, isolated from diabetic Goto-Kakizaki (GK) and Wistar (control) rats with different ages (6, 12, 26, and 52 weeks). We used a kinetic approach known as "top-down" analysis, which conceptually divides the oxidative phosphorylation system into two subsystems: one producing the protonmotive force (Δp) and another that consumes Δp. The overall response of the Δp generators to Δp was obtained from an uncoupler titration of respiration rate versus Δp, while the overall response of Δp consumers to Δp was obtained from an inhibitor titration of respiration rate versus Δp.

Our results showed that GK liver mitochondrial preparations presented an increase in Δp production and phosphorylative subsystems (using succinate as respiratory substrate). The alterations observed may suggest the existence of biochemical compensatory mechanisms to type 2 diabetes mellitus in GK rats during their first year of life, in order to reduce the injury associated with the disease. Furthermore, we observed that liver metabolic efficiency of mitochondrial respiration declined with age, this decrease in respiratory activity being visible both in control and diabetic rats.

Keywords Goto-Kakizaki (GK) Rats, Type 2 Diabetes Mellitus, Liver Mitochondria, Redox Chain, Phosphorylative System, Metabolic Control Analysis, Top-Down Analysis.

Received 5 April 2005; accepted 27 June 2005.

We thank the Portuguese Foundation for the Science and Technology for their support (Praxis XXI/PSau/1/96). F.M.L.F. is a grant recipient (SFRH/BD/3247/2000).

Address correspondence to Carlos M. Palmeira, Center for Neurosciences and Cell Biology of Coimbra, Department of Zoology, University of Coimbra, 3004-517 Coimbra, Portugal. E-mail: palmeira@ci.uc.pt

Abbreviations used:

Δp, protonmotive force; $\Delta\Psi$, electric membrane potential; FCCP, carbonyl p-trifluoromethoxyphenylhydrazone; TPP^+, tetraphenylphosphonium ion.

INTRODUCTION

Diabetes mellitus is a complex and costly noncommunicable disease that can affect nearly every organ in the body with devastating consequences, such as nontraumatic lower extremity amputations, renal failure, and blindness in working-age adult people. It is also a major cause of premature mortality, cardiovascular disease, stroke, peripheral vascular disease, and congenital malformations (Engelgau and Geiss 2000).

Type 2 (or non-insulin dependent) diabetes mellitus is the most common form of diabetes, accounting for around 85% of cases, affecting 100 million people all over the world. The two most common characteristics of type 2 diabetes mellitus (chronic hyperglycaemia and glucose intolerance) are associated with several metabolic derangements, but all patients present two common features: insulin resistance (i.e., ineffective use of insulin in target tissue) and beta cell secretory dysfunction (Engelgau and Geiss 2000). Since there is also an increasing prevalence, early interventions, reversal, and prophylaxis (when possible) of the disease are of major importance.

It has been established that diet, environment, and genetic susceptibility are important etiological factors for human type 2 diabetes mellitus. However, due to the polygenic nature of the disease, it becomes difficult to determine factors involved in the development of the disease. Therefore, animal models of diabetes are of great importance in the search for effective interventional treatment in type 2 diabetes mellitus, as experiments involving tissue sampling for assessment of

specific biochemical, metabolic, hormonal, and morphological parameters are not possible in humans (McIntosh and Pederson 1999).

In these studies, we used Goto-Kakizaki (GK) rats (a diabetic animal model, developed by Goto and Kakizaki) produced by selective breeding after 30 generations starting from a nondiabetic Wistar colony (Goto et al. 1975). GK rats exhibit moderate but enhanced fasting glucose levels, evident from 6 weeks of age, due to decreased beta cell secretion of insulin (Ostenson et al. 1993). Also, GK rats present normal lipidemia, peripheral and hepatic insulin resistance, and late complications, such as nephropathy or neuropathy. Furthermore, the GK rat is one of the best-characterized animal models of spontaneous nonobese type 2 diabetes mellitus, since it exhibits similar metabolic, hormonal, and vascular disorders to the human disease (McIntosh and Pederson 1999).

Diabetes mellitus appears to increase oxidative stress (Packer 1998). The increase in radical species generated can affect mitochondrial electron transport chain complexes and decrease coenzyme Q_{10} levels (Fosslien 2001). Moreover, increased oxidative stress also promotes membrane leakage. As a result, during the disease, ATP synthesis by F_0F_1ATPase seems to be decreased.

In the present paper, we investigate the possible alterations in liver mitochondrial respiratory system of GK rats during the first year of age, using an approach known as "top-down" analysis (Brand 1990). In this context, the oxidative phosphorylation system is conceptually divided into two subsystems: one producing the protonmotive force (Δp) (respiratory chain, succinate dehydrogenase, and dicarboxylate carrier) and another that consumes the Δp (adenine nucleotides and phosphate carriers, ATP synthase, and proton leak) (Hafner et al. 1988). These two component systems interact via Δp, which has been assumed to be entirely delocalized. The overall response of the Δp generators to Δp can be obtained from an uncoupler titration of respiration rate versus Δp; the overall response of Δp consumers to Δp can be obtained from an inhibitor titration of respiration rate versus Δp (Hafner et al. 1988).

EXPERIMENTAL PROCEDURES

Animals

Male spontaneously diabetic GK rats were obtained from a local breeding colony (Animal Research Center Laboratory, University Hospitals, Coimbra), established in 1995 with breeding couples from the colony at the Tohoku University School of Medicine (Sendai, Japan; courtesy of Dr. K. Susuki). Control animals were nondiabetic male Wistar rats of similar age, obtained from our local colony (Animal Research Center Laboratory, University Hospitals, Coimbra). Animals were kept under controlled light and humidity conditions and with free access to powdered rodent chow (diet C.R.F. 20, Charles Rivers, France) and water. Glucose tolerance tests were used as selection index.

Materials

All reagents and chemicals used were of the highest grade of purity commercially available.

Preparation of Mitochondria

Wistar and GK rats were maintained ad libitum for at least 12 h, before being sacrificed by cervical displacement, according to a preestablished method (Gazotti et al. 1979) with slight modifications.

Homogenization medium contained 250 mM sucrose, 5 mM HEPES (pH 7.4), 0.2 mM EGTA, 0.1 mM EDTA, and 0.1% deffated BSA. EDTA, EGTA, and deffated BSA were omitted from the final washing medium and adjusted to pH 7.2. The mitochondrial pellet was washed twice, suspended in the washing medium, and immediately used. Protein was determined by the Bradford method, using BSA (bovine serum albumin) as a standard (Sedmak and Grossberg 1977).

Simultaneous Measurement of Respiration Rate and Membrane Potential

Oxygen consumption of isolated mitochondria was analyzed polarographically at 25°C with a Clark oxygen electrode (Estabrook 1967). Respiration rate was measured as described before (Ferreira et al. 1997) and calculated assuming an oxygen concentration of 450 nmol O/mL in the experimental medium at 25°C.

The mitochondrial transmembrane potential was estimated by calculating transmembrane distribution of TPP^+ (tetraphenylphosphonium) with a TPP^+ selective electrode prepared according to Kamo et al. (Ferreira et al. 1997), using a calomel electrode as the reference. TPP^+ uptake has been measured from the decreased TPP^+ concentration in the medium sensed by the electrode. The voltage response of the TPP^+ electrode to $\log[TPP^+]$ was linear with a slope of 59 ± 1, in good agreement with the Nernst equation. The TPP^+ electrode was inserted in the glass chamber of the oxygen electrode, enabling the simultaneous measurement of $\Delta\Psi$ and respiration rate, essential for titration experiments. All experiments were carried out in the presence of the ionophore nigericin (75 ng/mL) and 130 mM KCl. This eliminates the pH gradient across the mitochondrial inner membrane (Murphy and Brand 1987) and therefore $\Delta\Psi$ is equal to Δp.

Determination of Adenine Nucleotides

Adenine nucleotides (ATP, ADP, and AMP) were extracted using an acidic extraction procedure and were separated by reverse-phase liquid chromatography, as previously described (Ferreira et al. 1997).

Statistics

The results are presented as mean $\pm$ SEM of the number of experiments indicated on the legends of the figures. Statistical significance was determined using unpaired Student's *t*-test and by using the one-way ANOVA Student-Newmann-Keuls

TABLE 1
Relationship between duration of diabetes and blood glucose levels both in Wistar (control) and in Goto-Kakizaki (GK) rats

Condition	Wistar	GK
6 weeks	108.57 ± 10.458	155.50 ± 17.457
12 weeks	118.17 ± 3.135	156.67 ± 10.535*
26 weeks	93.83 ± 1.424	212.00 ± 20.536***
52 weeks	93.67 ± 4.724	300.00 ± 25.485**

Blood glucose was evaluated at the time of animals' death, using a glucose oxidase test (Glucometer-elite, Bayer S.A., Portugal) and values are expressed as mg glucose per deciliter of blood.

Diabetic GK rats presented blood glucose levels significantly different from controls, from 12 to 52 weeks. Data are mean ± SEM of six-eight different blood analyses. Values statistically different from control: $^{*}p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.0005$.

posttest for multiple comparisons. For both tests P values <0.05 were considered significant.

RESULTS AND DISCUSSION

In the present study, we examined the effects of diabetes mellitus on mitochondrial bioenergetics. We used mitochondria isolated from liver of diabetic GK rats, an animal model of genetically nonobese type 2 diabetes, since liver plays an important role in the control of blood glucose levels, and like the beta cell, liver is highly exposed to glucose injury ("glucose toxicity"). Thus, impairment of liver bioenergetic functions, leading to a decrease in ATP contents, will be the main cause of glycemia level increase, since metabolism of glucose within the cells is highly dependent on the cellular pool of ATP (Gerbitz et al. 1996).

GK rats used in our studies exhibit moderate but enhanced fasting glucose levels, evident from 6 weeks of age. In fact, blood glucose levels, determined immediately after the sacrifice of animals, were significantly higher in GK rats as compared to Wistar rats (Table 1). Until the age of 12 weeks GK rats maintained a mild hyperglycemia; however, at 26 weeks of age the blood glucose levels increased substantially to values over than 210 mg glucose per deciliter of blood.

In the present study, we employed the "top-down" analysis approach, developed by Brand (1990), in order to identify changes in the regulatory characteristics of mitochondrial oxidative phosphorylation. We can consider oxidative phosphorylation (i.e., state-3 respiration) to consist of two component blocks of reactions: those reactions that generate the protonmotive force (Δp): the respiratory chain, succinate dehydrogenase, and dicarboxylate carrier, and those that consume Δp: the phosphorylating system (ATP synthase, adenine-nucleotide translocator, phosphate transporter, and any reactions that may be present in the matrix, converting ADP into ATP) and proton leak (the passive permeability of mitochondrial inner membrane to protons) and also other nonphosphorylating ion fluxes that dissipate Δp [Marcinkeviciute et al. 2000]). Obviously, in the presence of oligomycin (i.e., state-4 respiration) the only subsystem consuming Δp is proton leak.

These two blocks of reactions (Δp producers and Δp consumers) interact via a metabolic intermediate: the protonmotive force, Δp, which we assume to be entirely delocalized (Hafner et al. 1990a, 1990b). In the presence of K^+ ions in the reaction medium and nigericin, an H^+/K^+ exchanger, the pH gradient (ΔpH) is dissipated, and Δp given as $\Delta\Psi$ plus ΔpH can be entirely measured as $\Delta\Psi$, the electric membrane potential. This simplification is valid as long as $\Delta\Psi$ is the major contributor to Δp (Brown et al. 1990).

In order to evaluate the possible differences on the Δp-generating subsystem (in the presence of rotenone and using succinate as respiratory substrate), nonphosphorylating mitochondria, isolated both from Wistar (control) and GK (diabetic) rats from 6 to 52 weeks of age, were titrated with FCCP, a respiratory uncoupler (Fig. 1). Succinate enters the mitochondrial matrix, in exchange for malate, on the dicarboxylate translocater. Reducing equivalents, derived from succinate oxidation through succinate dehydrogenase, enter the respiratory chain at coenzyme Q level. In these experiments rotenone was used to ensure that endogenous substrates did not supply reducing equivalents at the NADH-ubiquinone-oxidoreductase level.

Our results showed that Δp generators, in the presence of succinate, after 12 weeks of age, were stimulated in GK liver mitochondrial preparations, since the plot of mitochondrial respiration rate against Δp, obtained from FCCP titration (in the presence of oligomycin), lies above the plot obtained with nondiabetic rats. At 6 weeks of age, the uncoupler titrations for mitochondria under state-4 conditions, both from GK and Wistar rats, are superimposable, suggesting that at the onset of the disease Δp producers in GK liver mitochondria are not stimulated. However, for the same values of respiratory rate (evaluated experimentally as the amount of oxygen consumed per minute), GK mitochondria presented higher values of Δp (evaluated experimentally as $\Delta\Psi$). These results are in agreement with our previous studies (Ferreira et al. 1999), which reported a stimulation of a FAD-linked substrate respiration, from 8 to 52 weeks of age in mitochondrial preparations isolated from liver of GK rats. Accordingly to Lionetti et al. (1998), we also showed that liver metabolic efficiency of mitochondrial respiration declines with age (see Fig. 1), this decrease in respiratory activity being visible both in control and diabetic rats. Moreover, the decrease in ADP/O ratios (Table 2) indicates an age-related decline in metabolic efficiency.

Nonphosphorylating mitochondria (in the presence of oligomycin, to inhibit ATP synthase) can be considered as a simple system, formed by two components interacting via the protonmotive force (Δp): the Δp generators and proton leak. The kinetic response of the proton leak to its driving force (Δp) was measured as the relationship between

TABLE 2
Relationship between respiratory indexes (RCR and ADP/O) determined in Wistar (control) and in diabetic Goto-Kakizaki (GK) rats' liver mitochondrial preparations

	RCR		ADP/O	
Age	Wistar	GK	Wistar	GK
6 weeks	7.21 ± 0.467	8.75 ± 0.740	1.70 ± 0.026	$1.99 \pm 0.024^{*}$
12 weeks	$4.59 \pm 0.464^{\S\S}$	$7.58 \pm 0.841^{*}$	1.73 ± 0.053	$1.81 \pm 0.032^{**}$
26 weeks	$4.48 \pm 0.237^{\S\S}$	$6.01 \pm 0.462^{\#\#,*}$	1.67 ± 0.021	$1.80 \pm 0.040^{***}$
52 weeks	$4.31 \pm 0.660^{\S}$	$5.86 \pm 0.172^{\#,*}$	1.61 ± 0.022	$1.76 \pm 0.080^{*}$

ADP/O and respiratory control (RCR) ratios were evaluated in the presence of succinate as respiratory substrate. Values are given as mean ± SEM of six independent experiments performed with three different mitochondrial preparations. Values statistically significant: $^{*}p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.0001$, as compared to respective controls; $^{\S}p < 0.01$; $^{\S\S}p < 0.001$ as compared to 6-week Wistar rats; $^{\#}p < 0.01$; $^{\#\#}p < 0.001$ as compared to six weeks GK rats.

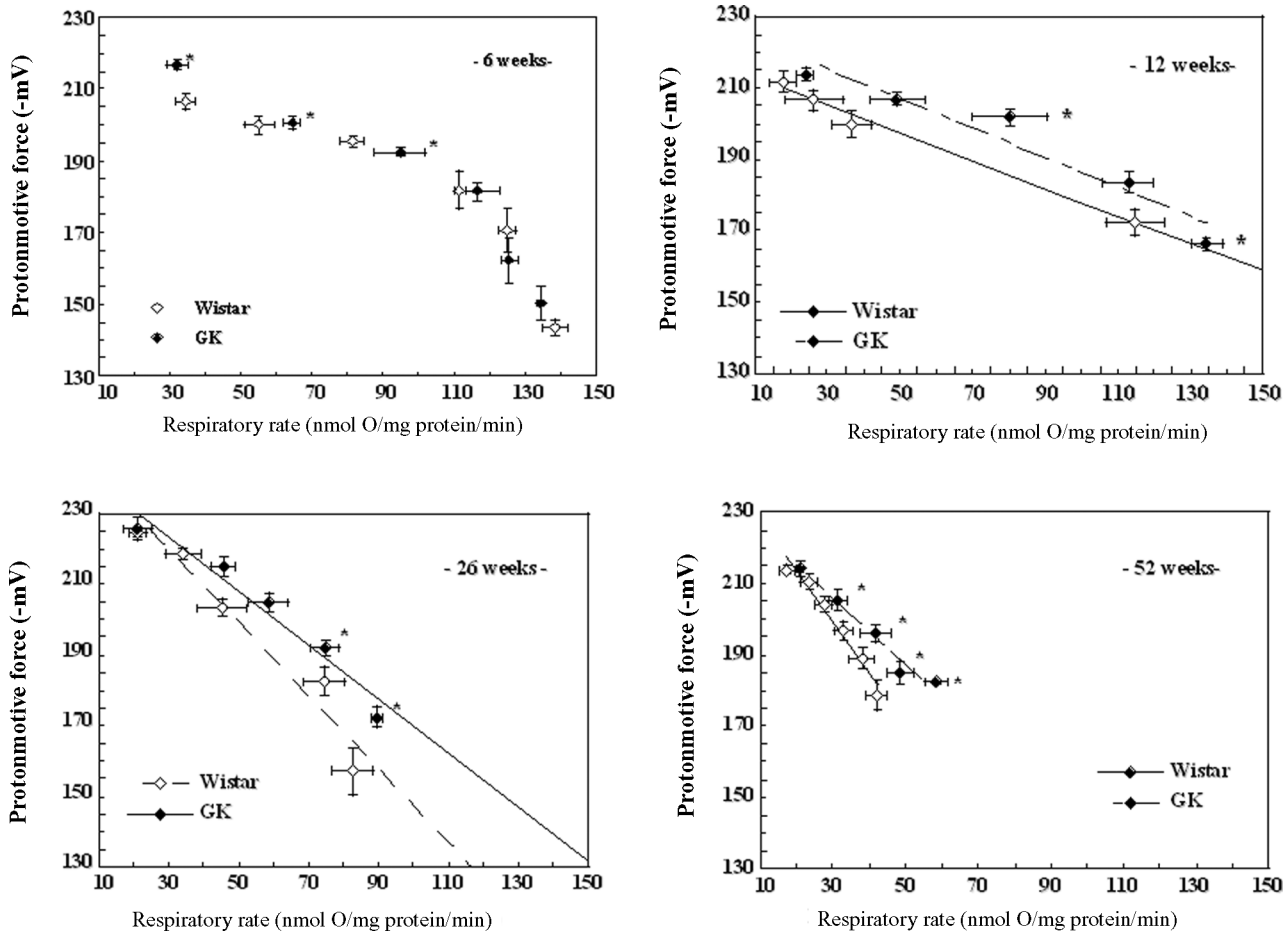

FIG. 1. FCCP titration of state-4 membrane potential and respiratory rate of liver mitochondria, isolated from diabetic GK and control rats.Nonphosphorylating mitochondria (with 1 μg/mL oligomycin added to standard respiration medium) isolated from Wistar (◇) and GK (◆) rats liver (with the ages indicated in each figure) were titrated with sequential additions of FCCP (10 nM). Respiratory rate and $\Delta\Psi$ were measured simultaneously, using sensitive oxygen and TPP^{+} electrodes, respectively. All experiments were carried out in the presence of the ionophore nigericin (75 ng/mL), to eliminate pH gradient across the mitochondrial inner membrane; therefore, $\Delta\Psi$ is equal to Δp. After each experiment, valinomycin (0,3 μg) was added for baseline corrections. Data are presented as means ± SEM of 6 independent experiments, performed with three different mitochondrial preparations. Values statistically significant: $^{*}p < 0.05$; $^{**}p < 0.005$, as compared to respective controls.

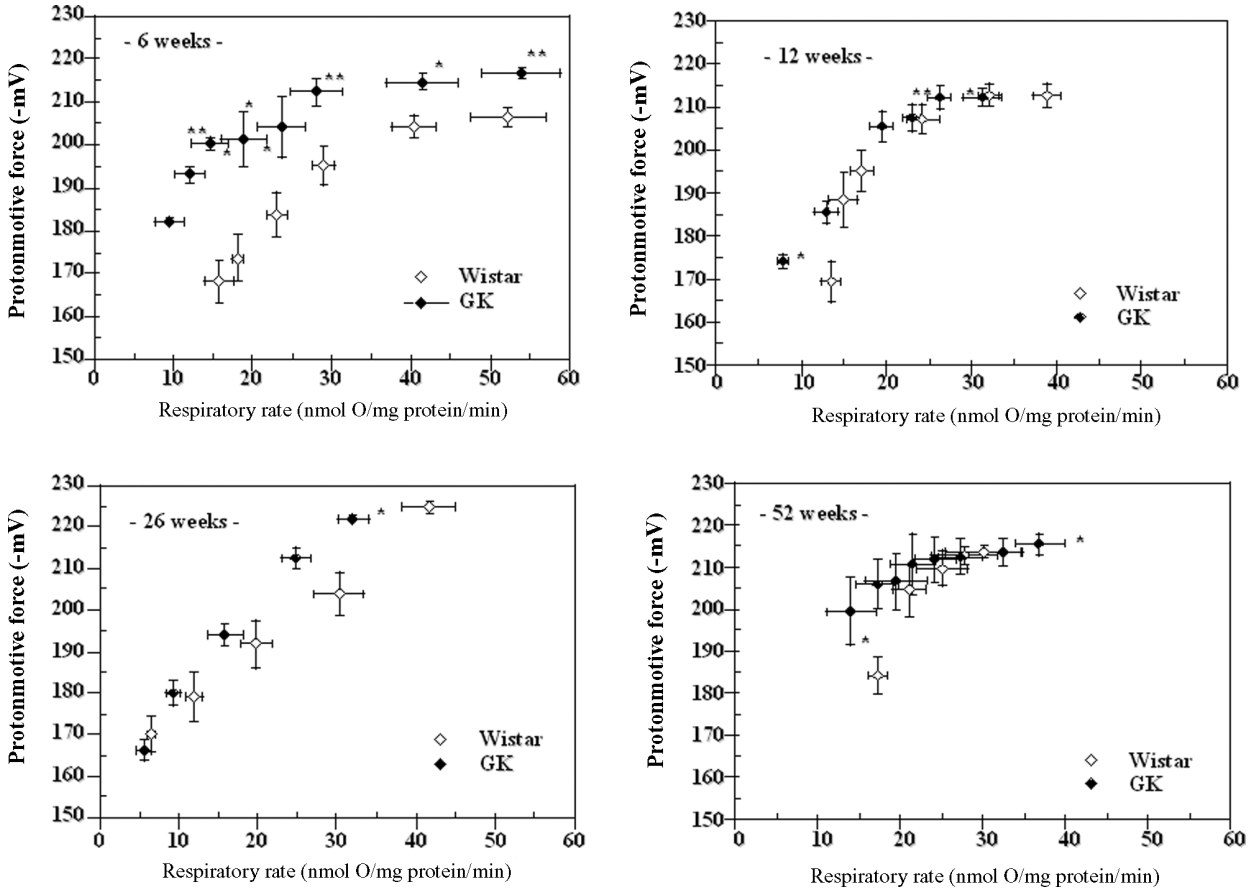

FIG. 2. Malonate titration of state-4 membrane potential and respiratory rate of liver mitochondria, isolated from diabetic GK and control rats.Nonphosphorylating mitochondria isolated from Wistar (◊) and GK (♦) rats' liver (with the ages indicated in each figure) were titrated with sequential additions of malonate (0,1 μM). Respiratory rate and $\Delta\Psi$ were evaluated as for Figure 1. Data are presented as means ± SEM of 6 independent experiments, performed with three different mitochondrial preparations. Values statistically significant: *$p < 0.05$; **$p < 0.005$, as compared to respective controls.

respiration rate and Δp (evaluated as membrane potential in the presence of K^+ ions and nigericin), when substrate oxidation reactions of state-4 mitochondria are progressively slowed by the addition of malonate, an electron transport inhibitor, since it interacts with succinate dehydrogenase. In our experiments, inhibition of the respiratory subsystem causes a decrease in $\Delta\Psi$; however, proton leak was not directly affected by these inhibitors, in the range of the concentrations used (0 to 300 μM) (Hafner et al. 1990a). Therefore, the respiration rate of mitochondria was proportional to the rate at which protons leak across the mitochondrial inner membrane (Hafner et al. 1990a).

If proton leak (a Δp consumer) is enhanced, then a plot of Δp against respiration rate, obtained from an inhibitor titration of state-4 respiration, will lie below the plot from control (Hafner et al. 1990b). Thus, our results clearly demonstrate that GK mitochondrial preparations presented a lower proton leak, since the plot of Δp against respiration rate from GK mitochondria lies above the plot from control, as shown in Figure 2. However, during the progression of diabetes, we observed a marked increase in proton leak from GK liver mitochondria. However, as occurrence of proton leak lowers ADP/O ratios (Brand et al. 1994), we observe a decrease in ADP/O ratio with age (see Table 2). These differences observed with mitochondrial preparations may have some important physiological consequences. The highest coupling between oxidative and phosphorylative systems observed in GK liver mitochondria can be a result of a biochemical adaptation to metabolic disturbances associated with type 2 diabetes mellitus. In fact, evidence indicates that the formation of free radicals in diabetic patients and in rats used as models of diabetes are increased (Knight 2000). Despite presenting mild hiperglycaemias, GK rats were exposed to a greater oxidative stress than control rats, since glucose, by a process of autoxidation in the presence of decompartmentalized trace transition metals, produces protein-reactive ketoaldehydes, hydrogen peroxide, and fragmentation of proteins (Trischler 1998). Thus, high glucose levels cause lipid and protein peroxidation. This fact may partially explain the highest increase in proton leak observed in mitochondria from GK rats from 6 to 52 weeks (more evident from 6 to 26 weeks), when compared to controls (see Fig. 2).

Phosphorylating mitochondria (without oligomycin added and in the presence of 3 mM ADP) were titrated with an inhibitor

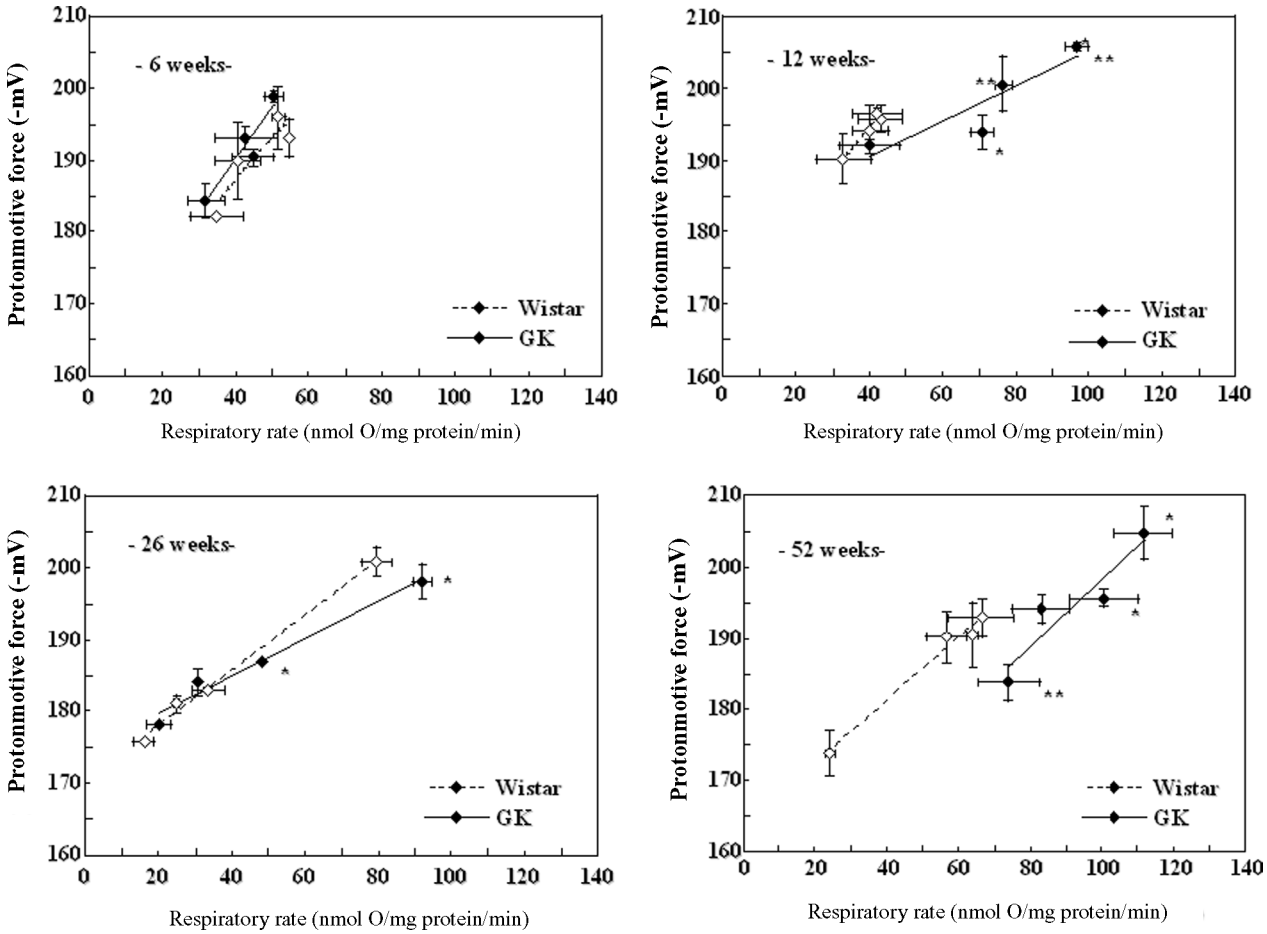

FIG. 3. Malonate titration of state-3 membrane potential and respiratory rate of liver mitochondria, isolated from diabetic GK and control rats.Phosphorylating mitochondria (without oligomycin added and in the presence of 3 mM ADP) isolated from Wistar (◊) and GK (♦) rats' liver with the ages indicated in each figure were titrated with sequential additions of malonate (0.1 μM). For further details see Figure 1. Data are presented as means ± SEM of 6 independent experiments for each malonate concentration used (0, 0.1, 0.2, and 0.3 μM) performed with three different mitochondrial preparations. Values statistically significant: $^{*}p < 0.05$; $^{**}p < 0.005$, as compared to respective controls.

of the respiratory chain (malonate), which enables manipulation of $\Delta\Psi$ (i.e., Δp because ΔpH was abolished in the presence of nigericin and K^+ ions), in order to evaluate the effects of diabetes and aging on Δp consumers (phosphorylative system and proton leak). Since proton leak through the mitochondrial inner membrane is strongly dependent on Δp, the plots obtained by titration of phosphorylating mitochondria with malonate can be easily transformed into proton current driving the phosphorylative system as a function of Δp (Murphy 1989).

Mitochondria isolated from 6-week-old GK rats showed a phosphorylating system less active than control rats of the same age, since the curve obtained for GK rats was displaced up and to the left, indicating that GK preparations presented a decrease in respiration rate at a given value of Δp. Nevertheless, at this age, GK rats showed a decrease in proton leak across the inner membrane, promoting also the observed displacement of the phosphorylating system titration curve.

Furthermore an apparent stimulation of the phosphorylative subsystem was observed in mitochondrial liver preparations of GK rats that were 12, 26, and 52 weeks-old (Fig. 3). The displacement of titration curves of diabetic rats downward (Hafner et al. 1990b) indicates that at any given value of $\Delta\Psi$ (Δp) during a malonate titration of state-3 respiration, mitochondria from GK rats presented a higher respiration rate than mitochondria from control rats. However, since these preparations also showed a decrease in proton leak, it becames difficult to evaluate the differences in phosphorylative subsystem of GK liver mitochondrial preparations, just by analyzing data from titration of mitochondrial state-3 succinate respiration.

Thus, with the purpose of evaluating the variations in ATP synthesis (and its dependence on Δp), we determined the amount of ATP produced upon titration with malonate and correlated it with Δp values (Fusi et al. 1992). The results presented in Figure 4 indicated that, in the absence of malonate, GK mitochondria developed a higher $\Delta\Psi$ and produced a greater amount of ATP than controls at the same age. Moreover, the observation that the amount of ATP produced in these experimental conditions increased with age and with the progression of diabetes indicates that in GK rats the liver mitochondrial functions

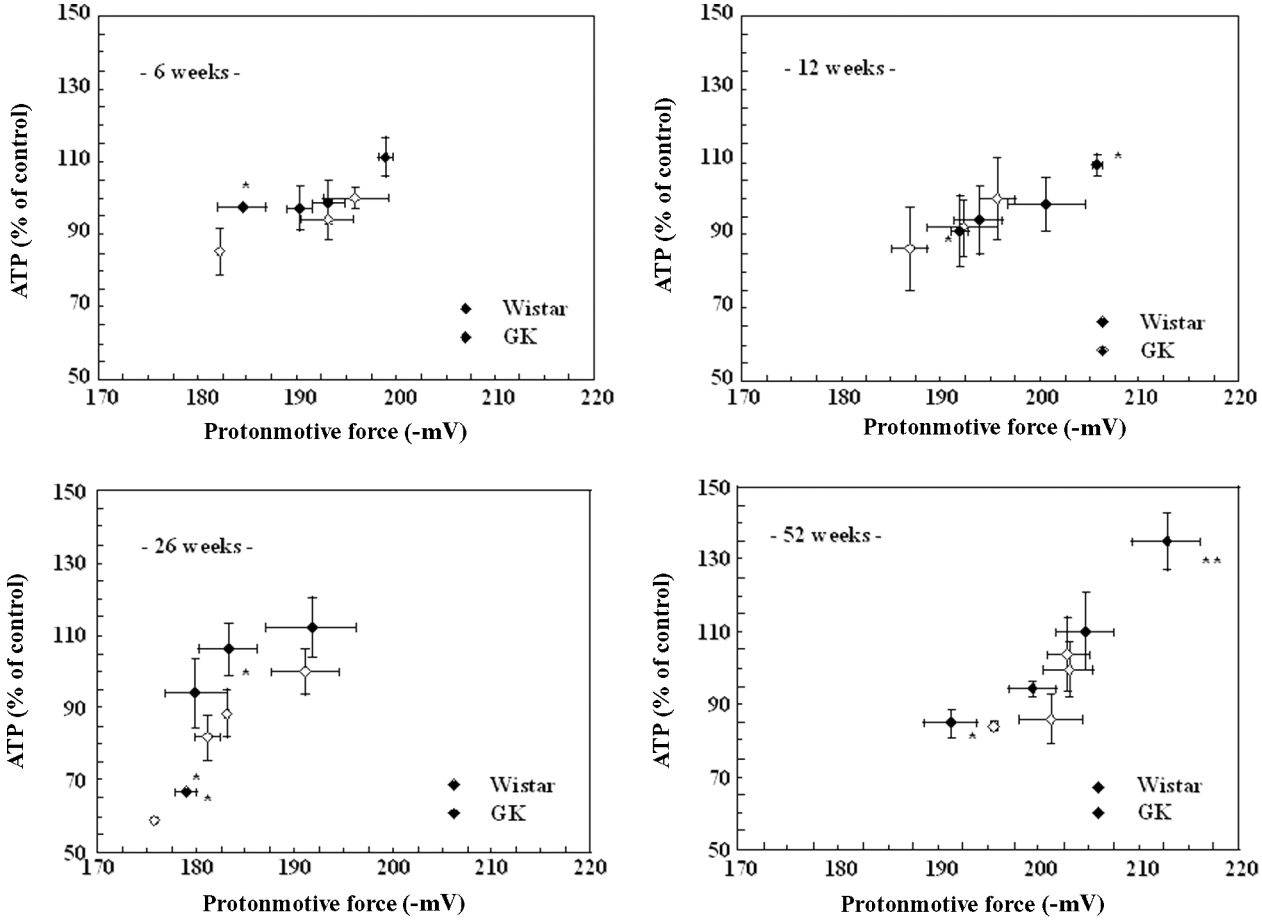

FIG. 4. Dependence of the ATP synthesis on the protonmotive force. Experimental conditions were similar to that described for Figure 3. After each experiment adenine nucleotides (ATP, ADP, and AMP) were extracted, using an acidic extraction procedure, and were separated by reverse-phase liquid chromatography. Results were expressed as a percent of the amount of ATP determined for Wistar preparations, without addition of malonate. Data are presented as means ± SEM of 6 independent experiments for each malonate concentration used (0, 0.1, 0.2, and 0.3 μM), performed with three different mitochondrial preparations. Values statistically significant: $^{*}p < 0.05$, as compared to respective controls.

were not impaired during their first year of life (Ferreira et al. 1999).

In summary, our results demonstrated that during the first year of life, the respiration of GK liver mitochondrial preparations using succinate as respiratory substrate was stimulated, probably reflecting an increased activity of succinate dehydrogenase. In addition, the phosphorylative subsystem of diabetic mitochondrial preparations also exhibited an increased activity. These modifications in oxidative phosphorylation in GK rats may correspond to an adaptative response of liver during the onset of type 2 diabetes mellitus, to exert protection against the cellular injury associated with the disease.

REFERENCES

Brand, M. D. 1990. The proton leak across the mitochondrial inner membrane. *Biochim. Biophys. Acta* 1018:128–133.

Brand, M. D., Chien, L.-F., Ainscow, E. K., Rolfe, D. F. S., and Porter, R. K. 1994. The causes and functions of mitochondrial proton leak. *Biochim. Biophys. Acta* 1187:132–139.

Brown, G. C., Hafner, R. P., and Brand, M. D. 1990. A "top-down" approach to the determination of control coefficients in metabolic control theory. *Eur. J. Biochem.* 188:321–325.

Engelgau, M. M., and Geiss, L. S. 2000. The burden of diabetes mellitus. In: Leahy, J. L., Clark, N. G., and Cefalu, W. T. (Eds.), *Medical Management of Diabetes Mellitus*, Dekker, Inc., New York, pp. 1–17.

Estabrook, R. E. 1967. Mitochondrial respiratory control and the polarographic measurement of ADP:O ratios. *Methods Enzymol.* 10:41–47.

Ferreira, F. M., Madeira, V. M., and Moreno, A. J. 1997. Interactions of 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene with mitochondrial oxidative phosphorylation. *Biochem. Pharmacol.* 53:299–308.

Ferreira, F. M., Seiça, R., Santos, M. S., and Palmeira, C. M. 1999. Age-related alterations in liver mitochondrial bioenergetics of diabetic Goto-Kakizaki rats. *Acta Diabetologica* 36:173–177.

Fosslien, E. 2001. Mitochondrial medicine—molecular pathology of defective oxidative phosphorylation. *Ann. Clin. Lab. Sci.* 31:25–67.

Fusi, F., Gsgaragli, G., and Murphy, M. P. 1992. Interactions of butylated hydroxyanisole with mitochondrial oxidative phosphorylation. *Biochem. Pharmacol.* 43:1203–1208.

Gazotti, P., Malmstron, K., and Crompton, M. 1979. In: Carafoli, E., and Sememza, G. (Eds.), *Membrane Biochem. A laboratory manual on transport and bioenergetics*, Springer-Verlag, New York, pp. 62–69.

Gerbitz, K. D., Gempel, K., and Brdiczka, D. 1996. Mitochondria and diabetes—genetic, biochemical, and clinical implications of the cellular energy circuit. *Diabetes* 45:113–126.

Goto, Y., and Kakizaki, M. 1975. Spontaneous diabetes produced by selective breeding of normal Wistar rats. *Proc. Jpn. Acad.* 51:80–85.

Hafner, R. P., Brown, G. C., and Brand, M. D. 1990a. Analysis of the control of respiration rate, phosphorylation rate, proton leak rate and protonmotive force in isolated mitochondria using the "top-down" approach of metabolic control theory. *Eur. J. Biochem.* 188:313–319.

Hafner, R. P., Brown, G. C., and Brand, M. D. 1990b. Thyroid-hormone control of state-3 respiration in isolated rat liver mitochondria. *Biochem. J.* 265:731–734.

Hafner, R. P., Nobes, C. D., McGown, A. D., and Brand, M. D. 1988. Altered relationship between protonmotive force and respiration rate in non-phosphorylating liver mitochondria isolated from rats of different thyroid hormone status. *Eur. J. Biochem.* 178:511–518.

Knight, J. A. 2000. The biochemistry of aging. *Adv. Clin. Chem.* 35:1–62.

Lionetti, L., Iossa, S., Liverini, G., and Brand, M. D. 1998. Changes in the hepatic mitochondrial respiratory system in the transition from weaning to adultwood in rats. *Arch. Biochem. Biophys.* 352:240–246.

Marcinkeviciute, A., Mildaziene, V., Crumm, S., Demin, O., Hoek, J. B., and Kholodenko, B. 2000. Kinetics and control of oxidative phosphorylation in rat liver mitochondria after chronic ethanol feeding. *Biochem. J.* 349:519–526.

McIntosh, C. H. S., and Pederson, R. A. 1999. Noninsulin-dependent animal models of diabetes mellitus. In: McNeill, J. H. (Ed.), *Experimental Models of Diabetes*, CRC Press, Boca Raton, Florida, pp. 337–398.

Murphy, M. P. 1989. Slip and leak in mitochondrial oxidative phosphorylation. *Biochim. Biophys. Acta* 977:123–141.

Murphy, M. P., and Brand, M. D. 1987. The control of electron flux through cytochrome oxidase. *Biochem. J.* 243:499–505.

Ostenson, C. G., Khan, A., Abdel-Halim, S. M., Guenifi, A., Suzuki, K., Goto, Y., and Efendic, S. 1993. Abnormal insulin secretion and glucose metabolism in pancreatic islets from the spontaneously diabetic GK rat. *Diabetologia* 36:3–8.

Packer, L. 1998. Oxidative stress and antioxidants: the antioxidant network, -α-lipoic acid, and diabetes. In: Packer, L., Rosen, P., Tritschler, H. J., King, G. L., and Azzi, A. (Eds.), Marcel Dekker, Inc., New York, Basel, pp. 1–15.

Sedmak, J. J., and Grossberg, S. E. 1977. A rapid, sensitive and versatile assay for protein using Coomassie Brilliant Blue G250. *Anal. Biochem.* 79:544–552.

Trischler, H. J. 1998. Experimental diabetic neuropathy: oxidative stress and antioxidant therapy. In: Packer, L., Rosen, P., Tritschler, H. J., King, G. L., and Azzi, A. (Eds.), Marcel Dekker, Inc., New York, Basel, pp. 121–128.