Gender dimorphism in rat liver mitochondrial oxidative metabolism and biogenesis

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Gender dimorphism in rat liver mitochondrial oxidative metabolism and biogenesis. Am J Physiol Cell Physiol 289: C372–C378, 2005.—In the present study, we have investigated gender differences in rat liver mitochondrial oxidative metabolism. Total mitochondrial population (M) as well as the heavy (M1), medium (M3), and light (M8) mitochondrial fractions obtained by means of differential centrifugation steps at 1,000, 3,000, and 8,000 g, respectively, were isolated. Electron microscopic analysis was performed and mitochondrial protein content and cardiolipin levels, mitochondrial O2 flux, ATP synthase activity, mitochondrial membrane potential, and mitochondrial transcription factor A (TFAM) protein levels were measured in each sample. Our results indicate that mitochondria from females have higher protein content and higher cardiolipin levels, greater respiratory and phosphorylative capacities, and more-energized mitochondria in respiratory state 3. Moreover, protein levels of TFAM were four times greater in females than in males. Gender differences in the aforementioned parameters were more patent in the isolated heavy M1 and M3 mitochondrial fractions. The present study demonstrates that gender-related differences in liver mitochondrial function are due mainly to a higher capacity and efficiency of substrate oxidation, likely related to greater mitochondrial machinery in females than in males, which is in accord with greater mitochondrial differentiation in females.

energy metabolism; mitochondrial biogenesis; high-resolution respirometry; mitochondrial membrane potential; mitochondrial transcription factor A

ENERGY BALANCE DEPENDS on the mechanism that regulates and coordinates energy uptake and the different components of energy expenditure, including basal metabolism, physical activity, and facultative thermogenesis. Gender differences in energy expenditure of adult rats at the usual rodent housing temperature have been established. Females have greater global O2 consumption, which is indicative of a higher energy expenditure than that of male rats (48). Gender-related dimorphism in brown adipose tissue (BAT) thermogenic capacity also has been found whereby females have greater tissue recruitment, which is reflected mainly in their mitochondrial features. These could be responsible, at least in part, for the differences in energy expenditure found between genders (42, 45, 48). Although liver is a significant contributor to basal metabolism (44), few studies actually have shown gender-related differences. However, recent research has found higher intrinsically antioxidant capacity in liver mitochondria in females than in males (3).

There is increasing evidence that mitochondrial function may be important for the switch between normal and pathological status of organs and tissues (6), which plays a crucial role in the pathological progression of diseases such as cancer (13), diabetes mellitus type 2 (33), and inflammation, as well as in aging-related diseases (49), hence making mitochondria suitable targets for therapeutic strategies (53). However, the role of mitochondria in gender dimorphism in the different incidence of these pathologies has not yet been defined fully.

Differences in mitochondrial characteristics, which include changes in size, mass, number, and composition, imply variations in their biogenesis in both proliferation and differentiation processes (11). In this sense, mitochondrial transcription factor A (TFAM) plays a key role in mitochondrial biogenesis because it is basic to initiation of both replication and expression of mitochondrial DNA (mtDNA) (11, 34). It is important to point out that mtDNA replication can be initiated by small amounts of TFAM and remains highly active over a range of TFAM concentrations, whereas expression of mtDNA, which is related to mitochondrial differentiation, is activated only at high concentrations of TFAM (10, 12, 27).

Previous studies also have shown that total mitochondrial population from liver and other tissues may be resolved by differential centrifugation into several fractions that show different morphological and functional features (14, 16, 21, 26). A connection between the process of mitochondrial biogenesis and mitochondrial fractions has been suggested, which implies that the lightest mitochondria would act as transitional forms of the heaviest, more differentiated, ones (23, 26, 29). In fact, gender differences in BAT mitochondrial fractions have been found whereby females show a higher degree of mitochondrial differentiation, especially in the heavier fractions (22).

Taking this background into account, the aim of this study was to examine gender-related differences in rat total liver mitochondrial oxidative metabolism and biogenesis. We have analyzed several features of liver total mitochondrial population, as well as mitochondrial fractions, a tool of mitochondrial biogenesis study. This study may be important to the further understanding of whether mitochondria are involved in the different incidence of several pathologies between the sexes.

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MATERIALS AND METHODS

Reagents and chemicals. All enzymes, substrates, and coenzymes were obtained from Sigma-Aldrich (St. Louis, MO) and Roche (Basel, Switzerland). Routine chemicals were supplied by Amersham Pharmacia Biotech (Little Chalfont, UK), Panreac (Barcelona, Spain), Sigma-Aldrich, Roche, and Bio-Rad (Hercules, CA). 10-Nonylhydroxystearic acid (NAO) and perchlorate of tetramethylrhodamine methyl ester (TMRM) mitochondrial probes were supplied by Molecular Probes (Eugene, OR). Polyclonal TFAM antibodies from rabbit were generously donated by Dr. Hidetoshi Inagaki (National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan).

Animals and mitochondria isolation. Animal experiments were performed in accordance with general guidelines approved by our institutional ethics committee and European Union rules 86/609/ECC. Wistar rats (110 days old; 13 males and 13 females) were supplied by Harlan (Barcelona, Spain). The animals were acclimated to 22°C and a 12:12-h light-dark cycle (lights on at 0800 h) and had free access to water and a standard chow diet (Panlab, Barcelona, Spain). All animals had been weighed previously and were killed at the start of the light cycle. The liver from each animal was dissected, washed, and weighed. Tissue was minced and homogenized in Tris-sucrose buffer (in mM: 250 sucrose, 10 Tris, and 1 Na2-EDTA, pH 7.4) using a Teflon/glass homogenizer at 500 rpm. An aliquot of the homogenate was used for measuring both DNA and mtDNA content as described elsewhere (see Refs. 54, 23, respectively). The homogenate was cleared of nuclear and cellular debris by performing centrifugation at 800 g for 10 min at 4°C. To obtain whole liver mitochondrial population (M), an aliquot of the cleared homogenate was centrifuged at 8,000 g for 10 min at 4°C. To isolate mitochondrial fractions, the rest of the cleared homogenate was subjected to three sequential centrifugation steps for 10 min at 1,000, 3,000, and 8,000 g to produce the heavy (M1), medium (M3), and light (M8) mitochondrial fractions, respectively. Pellets were resuspended in a suitable buffer, depending on the requirements of the analysis. Protein content was measured in each sample (4), thus providing a controlled protein load in cytometry, respirometry, and Western blot analysis.

ATP synthase activity. Mitochondrial ATP synthase (EC 3.6.1.3) activity was measured spectrophotometrically in each sample following the decrease in absorbance at 340 nm of NADH by coupling the decrease in absorbance at 340 nm of NADH by coupling the oxidation of NADH (43). The final volume of the reaction mixture was 1 ml and contained 0.950 μl of reaction buffer (in mM: 250 sucrose, 10 Tris, and 1 Na2-EDTA, pH 7.4) using a Teflon/glass homogenizer at 500 rpm. An aliquot of the homogenate was used for measuring both DNA and mtDNA content as described elsewhere (see Refs. 54, 23, respectively). The homogenate was cleared of nuclear and cellular debris by performing centrifugation at 800 g for 10 min at 4°C. To obtain whole liver mitochondrial population (M), an aliquot of the cleared homogenate was centrifuged at 8,000 g for 10 min at 4°C. To isolate mitochondrial fractions, the rest of the cleared homogenate was subjected to three sequential centrifugation steps for 10 min at 1,000, 3,000, and 8,000 g to produce the heavy (M1), medium (M3), and light (M8) mitochondrial fractions, respectively. Pellets were resuspended in a suitable buffer, depending on the requirements of the analysis. Protein content was measured in each sample (4), thus providing a controlled protein load in cytometry, respirometry, and Western blot analysis.

Respirometry. Mitochondrial O2 consumption was measured using the high-resolution Oxygen Graph respirometer (Oroboros, Austria) (15). The respiration medium consisted of 200 mM sucrose, 3 mM MgCl2, 0.5 mM EGTA, 0.1% BSA, 20 mM HEPES, 20 mM taurine, and 10 mM KH2PO4, adjusted to pH 7.1 with KOH at 37°C. The medium had previously been equilibrated with air in each chamber (set at 2 ml) at 37°C and stirred at 750 rpm until a stable signal was obtained for calibration at air saturation. A final concentration of 100 μg of total protein content per milliliter of respiratory buffer was used for the measurements. A protocol was developed for the measurements of the different mitochondrial chain respiratory states as follows: mitochondrial respiratory state 4 was measured in the presence of 5 mM succinate, active respiration (state 3) was then activated using 1 mM ADP, ATP synthases were inhibited by the addition of 1 μM/μl respiratory medium oligomycin (state 4o), and uncoupled respiration (state 3u) was provoked by the addition of 1 μM uncoupler carbonyl cyanide p-trifluoromethoxyphenyl-hydrazone (FCCP). Finally, mitochondrial respiration was stopped using 5 μM antimycin A. The titration protocol, which was completed within 50–60 min, was recorded at 2-s intervals using a computer-driven data acquisition system (Datlab, Austria).

Flow cytometry. Mitochondrial samples were diluted in a suitable respiratory buffer (220 mM sucrose, 20 mM glucose, 11 mM MgCl2, 3 mM HEPES, 2 mM KH2PO4, 0.5 mM EGTA, and 0.5% BSA, pH 7.4) to a final concentration of 100 μg of total protein content per milliliter. Mitochondria were activated to respiratory state 3 by the addition of both 5 mM succinate and 1 mM ADP. To measure mitochondrial membrane potential, activated mitochondria were incubated with 400 mM TMRM (Molecular Probes) for 15 min at 37°C. For mitochondrial cardiolipin measurement, activated mitochondrial samples were incubated with 1 μM of NAO (Molecular Probes) for 15 min at 37°C. Samples were analyzed using a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ) equipped with a 15-mW, 488-nm Argon ion laser. The green fluorescence (FL1), used for detecting the NAO signal, was collected through a 530/30 band-pass filter. Regarding the TMRM signal, the orange fluorescence (FL2) was collected through a 585/42 band-pass filter. Data acquisition (10⁴ events for each sample) was performed using CellQuest software (Becton Dickinson).

Western blot analysis of TFAM. Ten micrograms of protein from mitochondrial samples were fractioned by performing SDS-PAGE on 15% polyacrylamide gel according to the method Laemmli (25) and electrotransferred onto a nitrocellulose filter as described elsewhere (41). Staining with Ponceau S was used to provide visual evidence of correct loading and electrophoretic transfer of proteins to the nitrocellulose filter. Blocking and development of the immunoblots were performed using an enhanced chemiluminescence Western blot analysis system (Amersham). Rabbit polyclonal antibodies against TFAM were used as primary antibodies. Bands in films were analyzed using scanning densitometry and quantified using Kodak 1D Image Analysis software. Autoradiograms of membrane proteins revealed a protein exhibiting an apparent molecular mass of 25 KDa.

Statistics. All data are presented as mean values ± SE. Total mitochondrial population (M) and mitochondrial fractions (M1, M3, and M8) were analyzed as independent groups. Differences between genders with respect to body and liver weights and parameters measured both in liver homogenate and total mitochondrial population were assessed using Student’s t-test. Correlations of body and liver weights were analyzed separately in male and female rats by performing simple linear regression analysis. Differences between gender and mitochondrial fractions were assessed using two-way ANOVA and least-significant differences (LSD) for post hoc comparisons (P < 0.05). Because mitochondrial area data do not follow a normal distribution, differences between mitochondrial fractions were assessed using a nonparametric Kruskal-Wallis test. All statistical analyses were performed with SPSS 11.0 for Windows software (SPSS, Chicago, IL).
RESULTS

General parameters. Male and female Wistar rats (age, 110 days) showed differences in body and liver weight as expected (Table 1). Relative liver weight was the same in both sexes, indicating a close relationship between body and liver weight in accordance with previous studies (2, 20). In addition, a positive correlation between body and liver weight in both male and female rats was found ($R^2 = 0.870$, $P < 0.021$ and $R^2 = 0.962$, $P < 0.003$, respectively). Total DNA content, which illustrates the cellular content, was similar in both genders. Mitochondrial DNA content was not significantly different between genders.

Mitochondrial respirometry. Mitochondrial respiratory states (expressed as pmol $O_2$·s$^{-1}$·g of tissue$^{-1}$) were measured using high-resolution respirometry in total hepatic mitochondrial population (M) from male and female rats (Fig. 1). $O_2$ consumption in the presence of succinate (mitochondrial respiratory state 4) did not show significant differences between genders. No statistical differences in state 4 in the presence of oligomycin (state 4o), which allows respiration through proton conductance (proton leak) of the mitochondrial membrane, were found either. In contrast, $O_2$ consumption in state 3 (maximum ATP synthesis from ADP) was twice as high in females as in males. Uncoupled state 3 (provoked by the addition of FCCP (state 3u)) was almost identical to state 3, reflecting that the phosphorylation of ADP was not limited by the exchange of adenine nucleotides across the inner mitochondrial membrane under our experimental conditions. Furthermore, greater $O_2$ consumption in females in state 3u could indicate that female mitochondria have a higher content and/or activity of the electron transport mitochondrial chain (55).

Mitochondrial biochemical parameters. Protein content, ATP synthase activity, mitochondrial membrane potential, and cardiolipin levels of both mitochondrial population and mitochondrial fractions are summarized in Table 2. Although the protein content of mitochondrial population did not reach statistically significant differences between genders, the protein content of each mitochondrial fraction from females was significantly higher than the male counterparts, following the same tendency as the whole population. Regarding protein content distribution into mitochondrial fractions, both genders displayed a similar pattern in which M1 heavy fractions showed the highest values and the M8 light fractions showed the lowest.

To assess whether gender-related differences in $O_2$ consumption in respiratory state 3 was accompanied by differences in mitochondrial phosphorylative capacity, ATP synthase activity was measured. Female total mitochondrial population showed higher ATP synthase values (+33% compared with males). To conduct an exhaustive study of mitochondrial phosphorylative capacity, ATP synthase activity of each mitochondrial fraction also was analyzed. ATP synthase expressed per gram of tissue exhibited a profile similar to that of mitochondrial protein content, where the heaviest mitochondrial fractions are summarized in Table 2. Although the protein content of mitochondrial population did not reach significantly different values in state 4, the mitochondrial membrane potential and cardiolipin levels was set as 100%. Post hoc analysis for mitochondrial fraction using least-significant differences test and Student’s $t$-test.

Table 1. Gender differences in biometric parameters, DNA, and mtDNA content in rat liver

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>370±16</td>
<td>234±6*</td>
</tr>
<tr>
<td>Liver weight, g tissue</td>
<td>14.1±1.3</td>
<td>9.06±0.54*</td>
</tr>
<tr>
<td>mg tissue/g animal</td>
<td>38.1±0.8</td>
<td>38.7±0.7</td>
</tr>
<tr>
<td>mg DNA/g tissue</td>
<td>5.78±0.18</td>
<td>5.69±0.36</td>
</tr>
<tr>
<td>µg mtDNA/g tissue</td>
<td>4.80±0.84</td>
<td>3.54±0.57</td>
</tr>
</tbody>
</table>

Values are means ± SE of 13 animals per group. mtDNA, mitochondrial DNA. *$P < 0.05$, male vs. female (Student’s $t$-test).

Table 2. Gender differences in rat liver mitochondrial protein content, ATP synthase activity, membrane potential, and cardiolipin levels

<table>
<thead>
<tr>
<th>Mitochondrial protein content, mg/g tissue</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>13.4±1.7</td>
<td>17.7±3.0</td>
</tr>
<tr>
<td>M8</td>
<td>1.11±0.18</td>
<td>1.52±0.12</td>
</tr>
<tr>
<td>M3</td>
<td>2.65±0.30</td>
<td>3.71±0.40</td>
</tr>
<tr>
<td>M1</td>
<td>4.41±0.39</td>
<td>5.83±0.72</td>
</tr>
</tbody>
</table>

ATP synthase activity, IU/g tissue

| M                                         | 2.58±0.23 | 3.43±0.31* |
| M8                                        | 0.564±0.053 | 0.750±0.052 |
| M3                                        | 0.786±0.068 | 1.03±0.11 |
| M1                                        | 1.33±0.08 | 1.34±0.11 |

Mitochondrial membrane potential, au/10⁴ events

| M                                         | 100±6    | 165±22*  |
| M8                                        | 41.6±2.0 | 66.3±6.4 |
| M3                                        | 173±6    | 247±22   |
| M1                                        | 278±10   | 361±20   |

Cardiolipin content, au/10⁴ events

| M                                         | 100±6    | 119±10   |
| M8                                        | 119±13   | 141±12   |
| M3                                        | 125±8    | 153±12   |
| M1                                        | 131±9    | 166±10   |

Values are means ± SE of 5 animals per group (ANOVA; $P < 0.05$). F, effect of fraction; G, effect of gender; G × F, interactive effect; IU, international units; au, arbitrary units. Total mitochondrial population (M) and mitochondrial fractions were analyzed as independent groups. Mean value of M male membrane potential and cardiolipin levels was set as 100%. Post hoc analysis for mitochondrial fraction using least-significant differences test and Student’s $t$-test for total mitochondrial population ($P < 0.05$). a, M1, M3 ≠ M8; b, M1 ≠ M3.
fractions (M1 and M3) showed the highest values. Furthermore, female mitochondrial fractions displayed higher phosphorylative capacity than those of males.

Mitochondrial membrane potential in respiratory state 3 was analyzed because there is a direct correlation between this parameter and mitochondrial energy state in active respiration (30, 50). Mitochondrial membrane potential per mitochondrion in the total mitochondrial population from females was greater than that of males (+65%). Both genders displayed a common pattern in mitochondrial fractions, similar to protein content distribution, with M1 being the most energized and M8 being the least. However, females showed higher mitochondrial membrane potential than males in each isolated fraction.

Mitochondrial functional differences (O2 consumption, phosphorylative capacity, and energy status) between genders could be linked to changes in mitochondrial composition. Because cardiolipin has great relevance to both mitochondrial structure and respiratory function (51), mitochondrial cardiolipin levels also were measured. Although the total mitochondrial population did not reach statistically significant differences between genders, cardiolipin per mitochondrion from females in mitochondrial fractions was higher than in males, following the same tendency as the total population.

On the whole, compared with data in males, female liver mitochondria display higher protein content and cardiolipin levels, accompanied by more-energized mitochondria and greater phosphorylative capacity. This points to a greater mitochondrial differentiation state in female rat liver mitochondria focused in the heaviest fractions.

**Mitochondrial area and morphology.** To assess whether functional features of mitochondrial fractions are linked to mitochondrial morphological differences, transmission electron microscopic analysis was performed (Fig. 2 and Table 3).

Table 3. **Gender differences in rat liver mitochondrial area**

<table>
<thead>
<tr>
<th>Mitochondrial area</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>M8</td>
<td>0.188±0.009</td>
<td>0.204±0.010</td>
</tr>
<tr>
<td>M3</td>
<td>0.323±0.010*</td>
<td>0.332±0.008*</td>
</tr>
<tr>
<td>M1</td>
<td>0.339±0.008a</td>
<td>0.361±0.010ab*</td>
</tr>
</tbody>
</table>

Mitochondrial mean area (μm2) of mitochondrial fractions (M1, M3, and M8, sedimented at 1,000, 3,000, and 8,000 g, respectively) was obtained by measuring 75–150 mitochondria from 3 animals per group. Kruskal-Wallis test (P < 0.05). *Male vs. female; **M1, M3 ≠ M8; *M1 ≠ M3.

Previous morphometric studies concerning liver mitochondrial subpopulations have established a decreasing size pattern parallel to the increasing gravitational force used to obtain each subpopulation (26). In accordance with these findings, the morphometric analysis in our present study showed a typical profile with regard to the mitochondrial size of the fractions in both genders in which the heavy fractions (M1 and M3) had a greater area than the light fraction (M8). All mitochondrial fractions from female rats were significantly different in size, whereas in males, the heavier M1 and M3 mitochondria were almost identical in size. In addition, the mitochondrial area of the M1 fraction in females was significantly greater than that in males. Bearing in mind that protein content was higher in the M1 fraction (Table 2), these data suggest higher protein content per mitochondrion in female M1 mitochondria than in the male counterparts. In addition, M1 mitochondria from male rats were similar in size to the M3 mitochondria obtained from female rats, indicating that the former are more dense than M3 female mitochondria, probably because of a higher protein content per mitochondrion. No clear differences between genders regarding mitochondrial morphology in a given fraction were found (Fig. 2).

**Mitochondrial transcription factor A.** Mitochondrial transcription factor A (TFAM) levels were measured (Figs. 3 and 4) as a mitochondrial differentiation marker (10). Both genders displayed a similar pattern of TFAM distribution. The M1 fractions showed the highest values, whereas the M8 fractions showed the lowest. However, females showed higher TFAM protein levels in total hepatic mitochondrial population, approximately four times greater than those in males. All mitochondrial fractions also showed higher levels in females than in males, especially in the M1 and M3 fractions. These data reflect that females, particularly regarding the M1 mitochondria, could be more highly differentiated.

**Fig. 2.** Representative electron microscopic photomicrographs of mitochondrial fractions [heavy (M1), medium (M3), and light (M8), sedimented at 1,000, 3,000, and 8,000 g, respectively] of male and female rat liver. Bar in bottom right image, 1 μm.

**Fig. 3.** Representative Western blot analyses of mitochondrial transcription factor A (TFAM) levels of male (top) and female (bottom) rat liver. Total mitochondrial population (M) and mitochondrial fractions M1, M3, and M8 TFAM blots are shown. Protein (10 μg) of each sample was loaded for TFAM analysis.
mitochondrial fraction (least-significant differences) and Student’s fraction; G, effect of gender; G×F, interactive effect. Post hoc analysis for total mitochondrial population (*male vs. female. au, arbitrary units. 

potential and/or elevated O2 consumption may result in elevated reactive oxygen species (31). Previous studies have demonstrated that female rat liver mitochondria in respiratory state 4 generate fewer reactive oxygen species than found in males (3). These results, together with our present data, suggest that the mitochondrial respiratory chain from females is more active than from males (3). These results, together with our present data, suggest that the mitochondrial respiratory chain from females is more active than from males (3). These results suggest that females have more highly differentiated mitochondria, which are likely linked to differences in mitochondrial differentiation as displayed through the mitochondrial fractions study.

It is well known that the mitochondrial membrane potential steady state is the result of a balance between three subsystems: substrate oxidation, proton leak, and phosphorylation (5, 18, 37). The greater mitochondrial membrane potential found in females than in males suggests sex-related differences in the contribution of one or more of these subsystems. Taking into account that phosphorylative capacity, measured according to ATP synthase activity, is higher in females than in males, gender differences in mitochondrial membrane potential could be a result of lower mitochondrial proton leak and/or higher mitochondrial substrate oxidation. However, the mitochondrial proton leak in the total hepatic mitochondrial population, estimated on the basis of O2 consumption in state 4o, was similar in male and female rats, whereas the enhanced respiratory capacity of female mitochondria, estimated on the basis of uncoupled respiration (state 3u), indicates that female rats have a higher substrate oxidation capacity than do males. It is important to point out that increased mitochondrial membrane potential and/or elevated O2 consumption may result in elevated reactive oxygen species (31). Previous studies have demonstrated that female rat liver mitochondria in respiratory state 4 generate fewer reactive oxygen species than found in males (3). These results, together with our present data, suggest that the mitochondrial respiratory chain from females is more efficient than that of males.

At the tissular level, changes in the oxidative capacity and cellular O2 consumption are due to differences in mitochondrial number, size, and density per cell as well as to changes in mitochondrial components and/or activity (36, 38, 52). Because mtDNA content, which could be a suitable marker of mitochondria number (9, 24), between the sexes was not different, increases in mitochondria number as the main cause of higher capacity of substrate oxidation in females can be ruled out. Furthermore, taking into account morphometric analysis of mitochondrial fractions, female rats displayed larger mitochondria than did males, suggesting a link to the greater substrate oxidation system. However, oxidative substrate capacity differences (2-fold in females) could not be explained only by morphological features. In addition, because male and female rats have the same number of cells per gram of tissue as estimated on the basis of total DNA content, the greater oxidative capacity in female rat liver mitochondria could be linked to a greater machinery per mitochondrion and therefore to higher mitochondrial differentiation.

Mitochondrial biogenesis is the combination of both proliferation and differentiation processes. Proliferation is an increase in the mitochondrial population, while differentiation can be defined as an improvement of the functional capabilities of preexisting mitochondria (22, 23, 28, 39, 40, 56). Specifically, differentiation in liver mitochondria is based fundamentally on the construction of an adequate respiratory chain to make the ATP synthesis mechanism as efficient as possible (1, 17, 19, 26, 32). It is known that differences in the composition and/or number of mitochondria imply a different pattern in mitochondrial biogenesis (11). Female liver mitochondria show higher TFAM levels, a marker of mitochondrial differentiation that implies more highly differentiated mitochondria than found in males. This finding has been related to greater transcription of mtDNA (12), which is reflected in the greater mitochondrial machinery described in females in the present study.

Mitochondrial fractions have been used as a tool with which to study mitochondrial biogenesis because it seems there can be a connection with mitochondrial differentiation processes, whereby heavier mitochondria are the differentiated forms of the lighter ones (14, 21, 26). In fact, our data in both sexes show that heavy M1 fractions contained larger mitochondria, accompanied by greater protein content and phosphorylative capacity (features of more-differentiated mitochondria), whereas the lightest fractions had the lowest values (features of less-differentiated mitochondria). Furthermore, TFAM levels, which were higher in the heaviest than in the lightest fractions, support the hypothesis of a higher degree of mitochondrial differentiation in heavy fractions and reinforce the link between fractions and mitochondrial differentiation. In fact, as was true of the total mitochondrial population, functional markers in the mitochondrial fractions were higher in the female rats than in their male counterparts. Therefore, mitochondrial fractions highlight differences in mitochondrial differentiation between the sexes.

Previous studies have demonstrated that female rats have a higher total energy expenditure than do males (48). Differences between the sexes regarding in BAT recruitment, in which females contain more highly differentiated mitochondria, also have been found (42, 45–48). Likewise, the gender-related differences of functional features in liver mitochondria demonstrated in the present study could play an important physiological role and also could be related to the higher energy expenditure previously found in females (48).
One of the main factors responsible for the differences described in the present study could be the hormonal milieu. Previous studies have described the possible role of thyroid hormones regarding liver function, because female rats are more efficient in hepatic 3', 3'-triiodo-l-thyronine generation from thyroxine than that of males (8). The influence of sex hormones, estrogens in particular, on mitochondrial liver structure and function also has been described (3, 7, 35). Thus a possible influence of both sex steroid and thyroid hormones in liver mitochondrial function cannot be ruled out. In this sense, further research must be performed to clarify the main factors involved in sex-related differences in mitochondrial function, because this could be important information with respect to the mechanisms responsible for the different incidence of pathologies between the sexes regarding mitochondrial dysfunction. This information is important to the ability to optimize gender-specific protocols for treatments that target mitochondria.

In conclusion, the present study demonstrates sex-related differences in hepatic mitochondrial function. These differences are focused on the greater machinery per mitochondrion in females compared with males, which could be linked to more highly differentiated mitochondria and therefore to differences in mitochondrial biogenesis between the sexes.

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GENDER DIMORPHISM IN RAT LIVER MITOCHONDRIA


