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Original Article

Gender-specific proteomic alterations in glycolytic and mitochondrial pathways in aging monkey hearts

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Abstract

We utilized proteomic techniques in a primate model (*Macaca fascicularis*) of aging to determine potential mechanisms to explain gender differences in protection of the aging heart. The majority of prior work in this field utilized rodent models, and importantly no prior study utilized a proteomic approach in the aging heart. We studied changes in proteins in seven monkeys in each group (young and old males and females (YMs, OMs, YFs, and OFs, respectively)), and used two-dimensional gel electrophoresis in combination with mass spectrometry in five monkeys in each group. We found decreases ($P < 0.05$) in the expression of key enzymes in glycolysis (e.g. pyruvate kinase, α-enolase, triosephosphate isomerase), glucose oxidation (e.g. pyruvate dehydrogenase E1 β-subunit), and the tricarboxylic acid (TCA) cycle (2-oxoglutarate dehydrogenase) in left ventricular (LV) samples from OM monkeys; these changes in glycolytic, glucose oxidation, and TCA enzymes were not observed either in YMs, YFs or OFs. We found additional gender differences in the reduced expression and function of proteins that are responsible for electron transport and oxidative phosphorylation in mitochondria only in hearts from OM monkeys, with corresponding decreased oxidation rates with NADH and ascorbate-*N,N,N′,N′′*-tetramethyl-*p*-phenylenediamine substrates. The changes in glycolytic and mitochondrial metabolic pathways in OM monkey hearts are similar to changes observed in hearts affected by diabetes or LV dysfunction, and could be involved in the mechanism for the cardiomyopathy of aging. The sparing of these changes in OF hearts could be involved in the mechanism mediating delayed cardiovascular risk in OFs.

Keywords: Gender; Aging; Proteomics; Mitochondria; Glycolysis

Abbreviations: TCA, tricarboxylic acid; YM, young male; YF, young female; OM, old male; OF, old female; BW, body weight; 2D GE, two-dimensional gel electrophoresis; LV, left ventricular; IEF, iso-electric focusing; PAGE, polyacrylamide gel electrophoresis; DE, differential expression; MALDI-TOF, matrix-assisted laser desorption ionization time of flight.

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1. Introduction

Most studies of aging have employed rodent models or human subjects. In investigations in rodent models, species differences [1] must be considered, whereas human studies are complicated by age-associated diseases, such as diabetes or atherosclerosis. We utilized a primate model of aging (*Macaca fascicularis*), which is phylogenetically close to humans and does not have the associated diseases of aging [2]. These monkeys were old, but not senescent, roughly equivalent to humans 60 years of age. Therefore, the changes we observed could be ascribed to the development of aging cardiomyopathy, rather than secondary to atherosclerosis with its attendant complications due to myocardial ischemia. One goal of the current investigation involved examination of gender differences with aging, which in humans is complicated by the greater prevalence of atherosclerosis in elderly males than females [3].

We utilized a non-biased proteomic approach in hearts from young and old male and female (YM, OM, YF, and OF, respectively) monkeys. After 2D gel comparison in the different groups of monkeys, about 20 proteins, which showed different expression among the groups, were identified by mass spectrometry. Many of these proteins pointed to significant gender differences in glycolytic and mitochondrial electron transport protein expression, and function. Accordingly, the focus of this investigation was to determine, by proteomic techniques, the changes with aging, and gender in proteins related to metabolic pathways, since these changes could contribute to the discrepancy in life expectancy and cardiovascular risk between females and males [3,4].

2. Materials and methods

2.1. Animals

YM (n = 7, aged 6.3 ± 0.4 years; body weight (BW) 5.5 ± 0.4 kg); OM (n = 7, aged 20.1 ± 0.5 years; BW 5.4 ± 0.4 kg), YF (n = 7, aged 6.5 ± 0.4 years; BW 3.6 ± 0.5 kg), and OF (n = 7, aged 21.2 ± 0.4 years; BW 3.4 ± 0.2 kg) monkeys (*Macaca fascicularis*) were studied. Five monkeys in each group were used for two-dimensional gel electrophoresis (2D GE) analysis, seven in each group for immunoblotting of complexes I–V in the electron transport system, and four in each group for the oxygen consumption assay. All experiments examined each of the four groups of monkeys. The monkeys were fed a standard primate diet as previously described [2,5]. All of the young monkeys were born in captivity enabling us to know their exact age. All old monkeys were feral animals captured at the age of 5–7 years old, and had been kept in captivity for 12–15 years. The age at the time of capture was estimated based on eruption of dentition, general appearance, sexual development, and BW. After anesthesia with pentobarbital sodium, 50 mg/kg, the left ventricular (LV) was removed and samples were frozen rapidly in liquid nitrogen, and stored at –80 °C until needed. In addition, aortic samples from these monkeys were examined histologically and there was no evidence for atherosclerosis. Blood glucose levels indicated there was no diabetes in these monkeys. This investigation conforms to the *Guide for the Care and Use of Laboratory Animals* (Department of Health and Human Services publication (NIH) No. 83–23, revised 1996).

2.2. Protein extraction

For total protein extraction, approximately 50–100 mg of LV tissue was homogenized in 7 M urea, 2 M thiourea, 4% CHAPS, 0.5% IPG-ampholytes pH 3–10, 1% Triton X-100, 1% dithiothreitol, and protease inhibitor cocktail (SIGMA) and centrifuged at 12,000 g for 30 min at 4 °C. Protein concentration was determined using the Bradford protein assay with BSA as a standard. The yield of total proteins extracted from YM, OM, YF, and OF was 9.6 ± 0.1%, 11.0 ± 0.5%, 8.1 ± 1.0%, and 9.9 ± 1.1%, respectively.

Mitochondrial membranes were isolated at 4 °C from LV tissue by differential centrifugation using a mitochondria isolation kit (SIGMA). The yield of total mitochondrial membrane proteins extracted from YM, OM, YF, and OF was 0.35 ± 0.03%, 0.38 ± 0.06%, 0.42 ± 0.04%, and 0.45 ± 0.02%, respectively.

As the mitochondria were isolated from frozen tissue, they were not coupled and functioned as a crude mitochondrial membrane preparation. This was verified by the lack of effect of uncoupler on the oxidation of substrates, and by the ability to directly oxidize exogenous NADH. Oxidation of NADH was maximal and not further increased by the addition of detergent. Isolated mitochondrial membranes were solubilized in the same buffer as described above for total protein extraction.

2.3. 2D GE

Following the protein assay, the same amount of protein from each sample was diluted into 185 μl with a rehydration buffer containing 1% dithiothreitol. The samples were then rehydrated overnight on 11 cm IPG strips (pH range 5–8 and 3–10) at 50 V on a Bio-Rad iso-electric focusing (IEF) cell. After rehydration was complete, IEF was performed using a Bio-Rad IEF cell with a programmed voltage gradient. Second dimension SDS polyacrylamide gel electrophoresis (PAGE) was performed on 12.5% polyacrylamide gel. After electrophoresis, the gels were fixed in a 40% ethanol and 10% acetic acid solution, and then stained overnight with SYPRO Ruby dye. The gels were then destained and scanned with a 29202D master imager (Amersham Biosciences, Switzerland).

2.4. Image analysis of 2D gel

Gel patterns were compared with Z3 2D gel image analysis system Version 3.0 (Compugen, Israel). Using Z3, the images in each group were layered and registered. Spots

were detected with parameters of minimum spot area 24, minimum spot contrast 5 and minimum confidence level 0.95. The spot matching procedure compares the expression of similar spots in the registered images. The ratio of expression of the spot pairs (the differential expression, DE) on matched 2D gels was calculated. Matched spots were quantified in duplicate gels for each monkey. DE more than two was considered significant upregulation and less than 0.5 considered significant downregulation. Protein spots that showed at least a 2-fold alteration in density (DE > 2 or < 0.5) were excised for further identification. Maximum fit calibration method was used for correcting the differences between the compared images, which was caused by variations in experimental conditions, to obtain more precise DE values.

2.5. Protein identification

2D gel spots of interest were excised, destained, and digested with modified trypsin overnight. In gel tryptic digests were extracted and analyzed by ABI Voyager DE Pro matrix-assisted laser desorption ionization time of flight (MALDI-TOF) and ABI 4700 MALDI-TOF/TOF mass spectrometers. Spectra were acquired and measured peptide masses were searched in the NCBlnr protein sequence database using the MS-Fit and ProFound search engines with a mass tolerance of 50 ppm and a required four peptide minimum match.

2.6. Western blot analyzes

Western blot analyzes were performed with monoclonal antibodies specific for pyruvate kinase (Nordic Immunological Lab, Netherlands) and for representative subunits of human mitochondrial respiratory complexes I–V (Molecular Probes Inc, Eugene, OR).

2.7. Oxygen consumption assays

Oxygen consumption assays were performed on Strathkelvin O_2 meter (Model 781/781b) with a Clark oxygen electrode in a water-jacketed microcell, magnetically stirred, at 37 °C. Data were analyzed by 949 O_2 consumption system software (Version 2.2), as described previously [6]. The assays were performed with equal amounts of mitochondrial protein in the presence of an uncoupler, FCCP (0.05 μM): (1) oxidation of NADH (1 mM), which could be fully inhibited by rotenone (1 μM); (2) oxidation of succinate (1 mM), which was inhibited with antimycin A (5 μM); (3) oxidation of ascorbate (5 μM) plus *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD, 0.1 mM), which was inhibited with KCN (700 μM). Western blotting of mitochondrial respiratory complex II was used to verify equal loading of mitochondrial proteins in this oxygen consumption experiment since complex II is not altered with age in our monkey model.

2.8. Statistics

Data are expressed as mean ± S.E. Comparison of the data between groups were performed by Student's paired test with significant differences taken at $P < 0.05$. For baseline data and oxygen consumption assays, a repeated one-way ANOVA with Student–Newman–Keul's test (SAS Institute: Cary, NC) was used. To quantitate differences in proteins identified in 2D gels, we used the standard approach of DE. The average DE and standard error of the mean from five monkeys in each group was calculated using Microsoft Excel. For increases to be significant, the difference had to be greater than 2-fold, and for decreases, the differences had to be greater than 2-fold, i.e. more than a 50% decrease.

3. Results

3.1. Gender-specific alterations observed from LV total protein extracts

A typical SYPRO Ruby stained 2D gel from LV total protein extracts (150 μg) of a YM monkey is shown in Fig. 1 (left panel). By 2D gel comparison, major differences in protein expression were found related to metabolic pathways. Circled spots indicate aging and gender-specific proteins in metabolic pathways, which was the focus of the current study. Enlargements of regions of 2D gel images containing aging and gender-specific differences in expression of metabolic enzymes are shown in Fig. 1 (right panel). Proteins involved in fatty acid metabolism were upregulated in both OM and OF. 3-Oxoacid CoA transferase increased by an average of 2.6-fold (DE = 2.6 ± 0.4) in the OM group compared with the YM group, similar increases were seen in the OF compared with the YF, and acyl-CoA dehydrogenase increased by an average of 2.2-fold (DE = 2.2 ± 0.3) in the OM group compared with the YM group and by an average of 4.1-fold (DE = 4.1 ± 0.4) in the OF group compared with the YF group.

Glycolytic proteins were downregulated only in OM (Table 1). For example, 60–70% reductions in triosephosphate isomerase (DE = 0.29 ± 0.08), α-enolase (DE = 0.28 ± 0.15), tricarboxylic acid (TCA) cycle protein 2-oxoglutarate dehydrogenase (DE = 0.33 ± 0.10) were observed in OM compared with YM. Western blotting was used to detect the levels of metabolic proteins (Fig. 2). The key glycolytic protein, pyruvate kinase, was significantly downregulated in the OM compared with YM and OF ($P < 0.05$), while there were no significant differences between the YF and OF. There were also no significant differences between YM and YF.

3.2. Gender-specific alterations in LV mitochondrial proteins

A 2D gel image of isolated mitochondrial proteins (150 μg) from an OM monkey LV is illustrated in Fig. 3 (left panel), with magnified regions containing aging and gender-specific differences in expression of metabolic enzymes (Fig. 3, right panel). The following proteins were reduced by

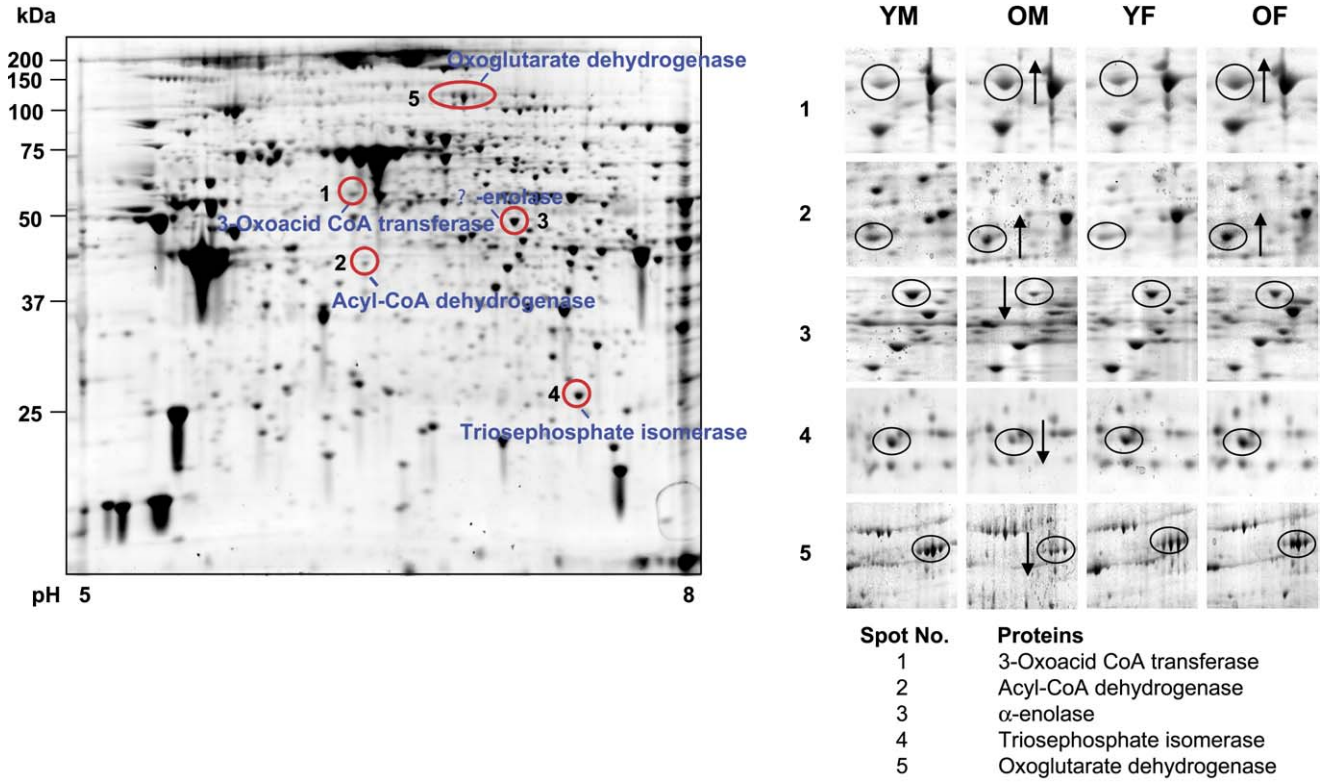

Fig. 1. 2D gel images containing aging and gender-specific differences in expression of metabolic enzymes from monkey LV total proteins. The left panel shows a 2D gel map of total LV proteins extracted from a YM monkey; 150 μg of protein was separated by 2D GE, stained with SYPRO Ruby, quantified by Z3 software and identified by MALDI-TOF mass spectrometry. The circled spots indicate altered proteins, which were identified and related to metabolic pathways. The right panel shows enlargements of regions of 2D gel images containing aging and gender-specific differences in expression of metabolic enzymes. The numbers 1–5 refer to proteins labeled in the left hand figure and shown at the right in the enlarged regions of the 2D gel image. 3-Oxoacid CoA transferase (1) and acyl-CoA dehydrogenase (2) were upregulated in both OM and OF monkeys compared to YM and YF monkeys. α-enolase (3), triosephosphate isomerase (4), and oxoglutarate dehydrogenase (5), were downregulated only in OM, but not in OF monkeys.

50–65% only in OM: pyruvate dehydrogense E1 β-subunit (DE = 0.49 ± 0.13), ATP-specific succinyl-CoA synthetase β-subunit (DE = 0.36 ± 0.11), and ATP synthase α-subunit involved in the electron transport system (DE = 0.49 ± 0.15).

In contrast, acyl-CoA dehydrogenase, which is involved in fatty acid metabolism, was increased in both OM and OF by 2.5-fold (DE = 2.5 ± 0.5), directionally consistent with the results from total protein extracts shown in Fig. 1.

Table 1
Alterations in protein expression related to metabolic pathways

Proteins	Accession no.	Metabolic pathway	OM vs. YM[a]	OF vs. YF[a]	Detection methods
3-Oxoacid CoA transferase	P55809	Fatty acid oxidation	↑	↑	2DGE(T,M)
Acyl-CoA dehydrogenase	P16219	Fatty acid oxidation	↑	↑	2DGE(T)
α-enolase	P06733	Glycolysis	↓	↔	2DGE(T)
Triosephosphate isomerase	P00938	Glycolysis	↓	↔	2DGE(T)
Pyruvate kinase	P14618 P14786	Glycolysis	↓	↔	WB
Pyruvate dehydrogense E1 β-subunit	P11177	Glucose oxidation	↓	↔	2DGE(M)
Oxoglutarate dehydrogenase	Q02218	TCA cycle	↓	↔	2DGE(T)
ATP-specific succinyl-CoA synthetase β-subunit	Q9P2RT	TCA cycle	↓	↔	2DGE(M)
ATP synthase α-subunit	P25705	ETS	↓	↔	2DGE(M), WB

2DGE(T): 2DGE of total protein extracts; 2DGE(M): 2DGE of isolated mitochondrial membranes; ETS: electron transport system; TCA: tricarboxylic acid; WB: western blotting.
↑: Indicates increased levels of the protein.
↓: Indicates decreased levels of the protein.
↔: Indicates unchanged levels of the protein.
[a] Average change fold was described in Section 3 of this article.

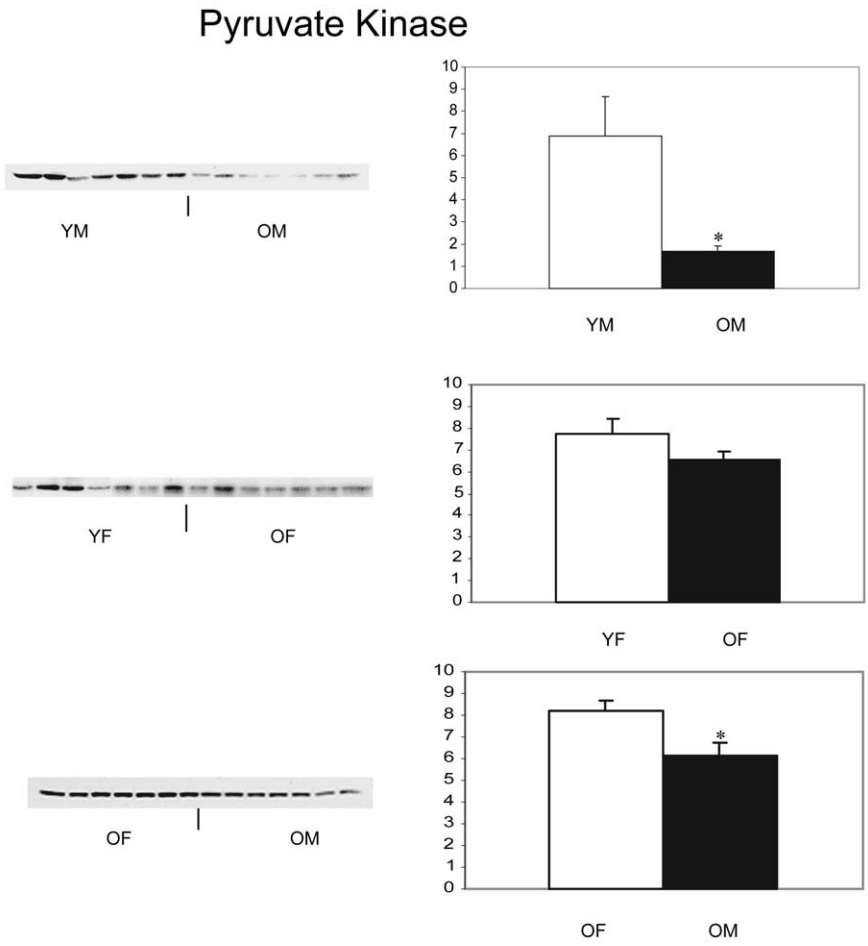

Fig. 2. Western analysis of pyruvate kinase in monkey LV. Total protein extracts (20 μg per sample) were subjected to SDS PAGE, electroblotted to nitrocellulose membrane, and probed with sheep polyclonal anti-pyruvate kinase antibody. Pyruvate kinase was clearly downregulated in the OM compared with the YM group ($P < 0.05$) (top). There was no significant difference between the YF and OF groups (middle). The bottom panels compare OM and OF and show that pyruvate kinase was significantly less in OM than OF. The asterisks indicate significantly different data, $P < 0.05$.

3.3. Gender-specific differences in proteins responsible for electron transport and oxidative phosphorylation in LV mitochondria

Since we found downregulation of the ATP synthase α-subunit only in the OM group by proteomics (Fig. 3), we hypothesized that there were gender differences in mitochondrial electron transport/respiration for ATP production. As shown in Table 2, mitochondrial proteins belonging to the energy-producing pathway: ubiquinol-cytochrome *c* oxidoreductase (complex III), cytochrome *c* oxidase (complex IV), and ATP synthase α, β, and d subunit (complex V) were significantly decreased only in aging male monkeys compared to YM ($P < 0.05$). There were no significant differences between YF and OF. For the comparison of OM vs. OF blots, we selected one subunit for each of the complexes I–IV and two subunits for complex V. Complexes III–V were decreased significantly in OM compared to OF monkeys (data not shown). For this latter comparison, the westerns were run again comparing OM vs. OF. Western analysis of the ATP synthase α-subunit is shown in Fig. 4. In the LV mitochondrial analysis the changes in 2-oxoglutarate dehydrogenase did not meet the 2-fold DE criterion.

3.4. Alteration in mitochondrial respiratory chain function in aging monkeys analyzed by oxygen consumption studies

A polarographic assay of oxygen consumption of the mitochondrial respiratory chain enzymes demonstrated that oxidation of NADH was reduced in the OM vs. YM (0.07 ± 0.02 vs. 0.29 ± 0.06 O_2 μmol/min/μg, $P < 0.05$). In contrast, OF was not different from YF (0.18 ± 0.06 vs. 0.24 ± 0.04 O_2 μmol/min/μg). Oxidation of ascorbate-TMPD, which feeds electrons directly to complex IV showed similar differences, i.e. OM vs. YM (0.14 ± 0.02 vs. 0.30 ± 0.02 O_2 μmol/min/μg, $P < 0.05$), while OF was not significantly different from YF (0.20 ± 0.02 vs. 0.16 ± 0.02 O_2 μmol/min/μg). Oxidation of succinate showed the same trend, i.e. decreased in OM, but not OF, but the data were not significant.

4. Discussion

The non-biased proteomic approach employed in this investigation uncovered major defects in energy-producing pathways, which are summarized in Tables 1 and 2. These

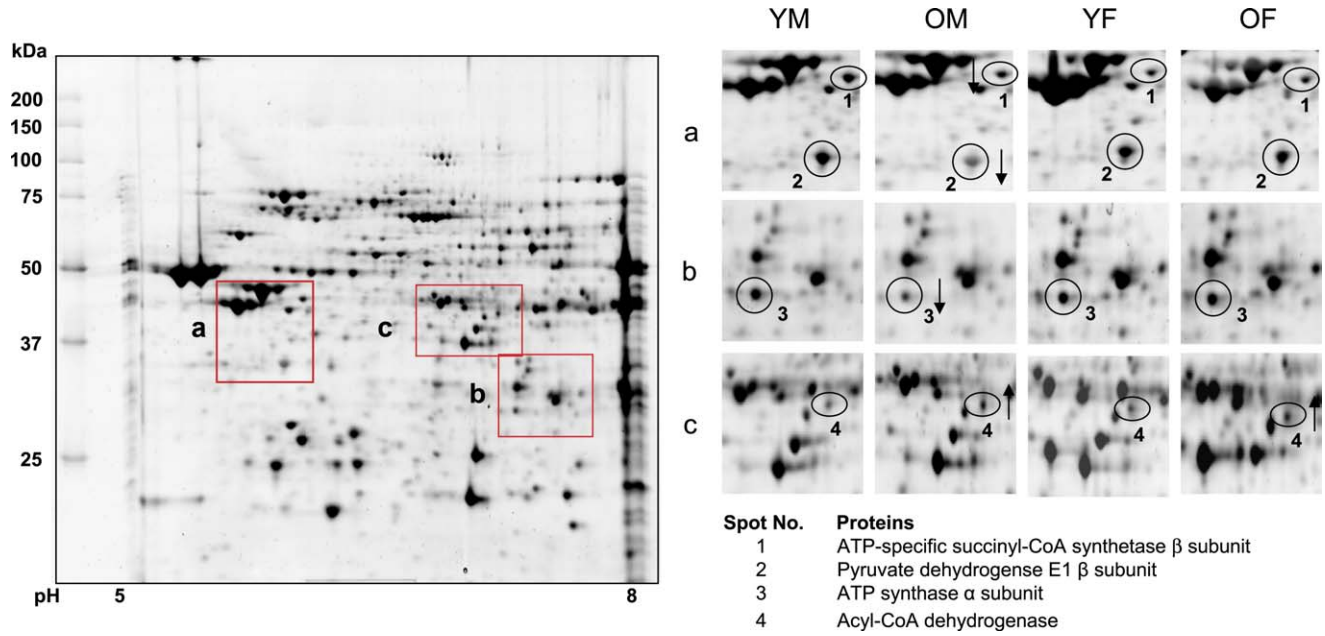

Fig. 3. 2D gel images containing aging and gender-specific differences in expression of metabolic enzymes of mitochondrial proteins isolated from monkey LV. The left panel shows a 2D gel map of mitochondrial proteins isolated from an OM monkey. Images a–c indicate areas containing proteins which were altered and identified as related to metabolic pathways. The right panel shows enlargements of regions of 2D gel images containing aging and gender-specific differences in expression of metabolic enzymes. The numbers 1–4 identify specific proteins that were altered. Acyl-CoA dehydrogenase (4) was upregulated in both OM and OF monkeys compared to YM and YF monkeys. Pyruvate dehydrogenase E1 β-subunit (2) and ATP synthase α-subunit (3) are downregulated only in OM, but not in OF. ATP-specific Acyl-CoA synthase β-subunit (1) was downregulated in OM vs. YM, but not in OF vs. YF.

results indicate that both OM and OF monkeys increase the level of fatty acid enzymes with aging. However, only OM monkeys showed a decreased expression of enzymes participating in glycolysis, glucose oxidation, the TCA cycle, and the electron transport system (complexes III–V). Furthermore, we demonstrate that the capacity of mitochondria for oxygen consumption is significantly reduced in OM monkeys, not in OF. The decline of protein levels and function in aging males could result in electron leakage during electron transport, generating more reactive oxygen species, which increases oxidative damage to the mitochondrial, cytosolic, and nuclear compartment, and will eventually lead to myocardial dysfunction and apoptosis [7–11]. OFs by maintaining the protein level, and function in electron transport may increase protection from reactive oxygen species, and further increase life span. Importantly, elevated ROS production is known to occur in aging animals, and complex III in the mitochondrial electron transport chain is a putative site for

Table 2
Gender and age related changes in mitochondrial complexes I-V [a]

		YM	OM	YF	OF
Complex I					
	30KD	6.86 ± 1.10	7.42 ± 0.92	7.05 ± 1.11	7.24 ± 0.71
	39KD	3.72 ±0.24	4.02 ± 0.16	2.76 ± 0.18	3.06 ± 0.21
Complex II					
	30KD	7.40 ± 0.54	6.88 ± 0.46	7.41 ± 0.67	6.87 ± 0.92
	70KD	4.13 ± 0.09	4.16 ± 0.20	2.71 ± 0.27	2.95 ± 0.14
Complex III					
	core 1	3.27 ± 1.80	1.81 ± 0.23 *,†	4.77 ± 0.44	4.04 ± 0.24
	core 2	8.54 ± 0.93	5.75 ± 0.86 *	7.87 ± 0.85	6.41 ± 1.08
Complex IV					
	subunit 1	5.13 ± 0.37	2.38 ± 0.31 *	3.66 ± 0.25	2.51 ± 0.53
	subunit 2	3.62 ± 0.38	2.01 ± 0.44 *,†	4.00 ± 0.12	4.06 ± 0.25
Complex V					
ATP synthase					
	α subunit	3.34 ± 0.78	0.82 ± 0.17 *,†	5.89 ± 0.95	4.21 ± 0.47
	β subunit	9.61 ± 1.39	4.68 ± 0.37 *,†	7.32 ± 0.54	6.96 ± 0.47
	d subunit	5.97 ± 0.68	2.10 ± 0.56 *	2.35 ± 0.89	1.81 ± 0.68

[a] Intensity of western blots was compared between OM and YM, between OM and YF, and between OM and OF. There were no significant differences between OF and YF, but significant differences, $p < 0.05$, were found between OM and YM, indicated by *, and between OM and OF indicated by †.

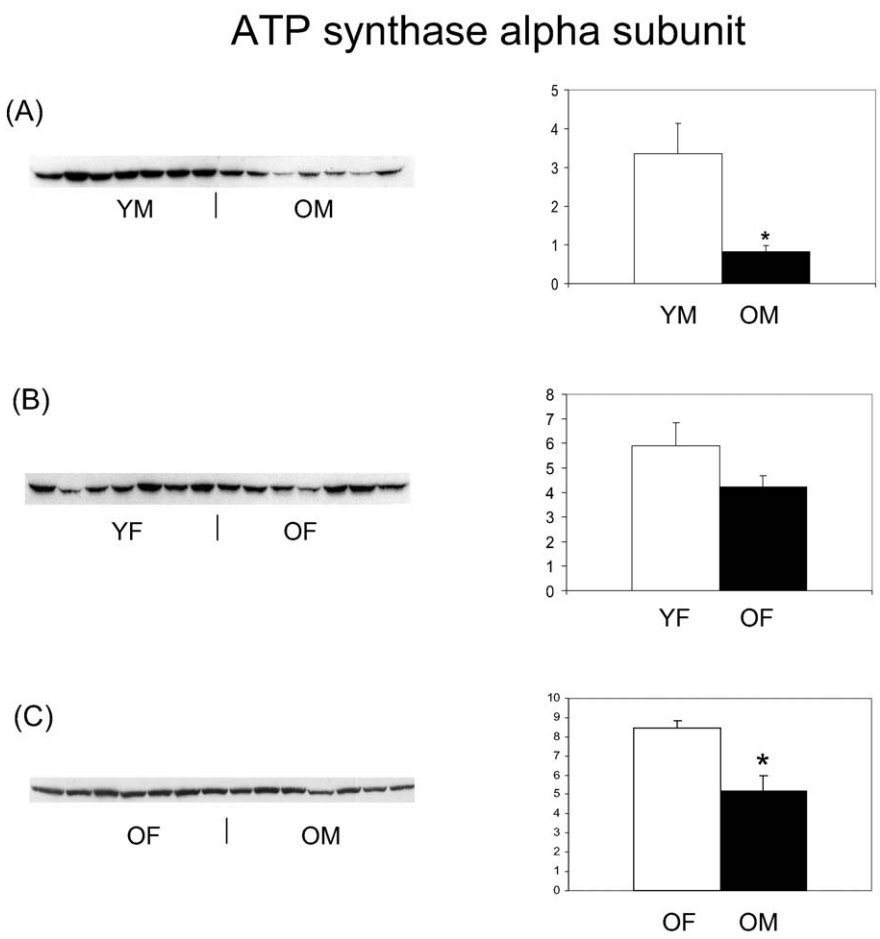

Fig. 4. Western analysis of ATP synthase α-subunit in monkey LV. The ATP synthase α-subunit was significantly decreased in OM compared to YM (top) but not significantly decreased in OF vs. YF monkeys (middle). This protein was also decreased in OM vs. OF (bottom). The asterisks indicate significant differences, $P < 0.05$.

the enhanced production of reactive oxygen species that contribute to aging in the heart [9]. Although we did not measure the level of increased ROS in older animals, the more pronounced dysfunction in complex III in OM could lead to more leakage of ROS into the mitochondria, and play a role in the subsequent decline in cardiac energetic function.

There are several possible mechanisms by which aging can decrease mitochondria levels in heart. First, mitochondrial DNA (mtDNA) deletions and mutations have been reported to occur with aging in various tissues [10]. Consequently non-functional proteins are expressed, while normal protein levels are decreased. Second, increased ROS results in loss of mitochondrial membrane potential and cardiac myocyte apoptosis [11]. Third, in aging animals both mitochondrial transcription and translation activities have been found to be impaired [12]. This phenomenon is also seen in nuclearly expressed proteins that are later transported into the mitochondria [13].

It is important to recognize that the monkeys used in the present study are old, but not senescent, and accordingly have not developed fully the cardiomyopathy of aging. However, we have reported that the OM monkeys exhibit more apoptosis than YM [14]. In addition, a recent study from our laboratory demonstrated that LV function in the OM monkeys was compensated at baseline, but was not able to respond appropriately to catecholamine stimulation compared with YM [15]. Thus, the changes in mitochondrial proteins observed in the current study may be responsible, in part, for these aspects of aging cardiomyopathy, which appear to precede the development of baseline LV dysfunction in OM monkeys.

Although no prior study utilized proteomic techniques in the heart, one report demonstrated the feasibility of using these techniques for the examination of aging in mitochondrial proteins in skeletal muscle [16]. However, several other studies found alterations in metabolic and mitochondrial pathways in the aging heart, examining different end-points [17–31], but the results of these studies were not always directionally similar. Several studies have suggested older animals increasingly utilize glycolysis for cardiac energy production [17], and have a decreased ability to oxidize fatty acids [17–19], others have found opposing results on glycolysis [20,21]. Several studies of the mitochondrial electron transport system for complex I [22–25], complex II [26], complex III [22,27,28], complex IV [18,22,23,28,29], and complex V [26] found decreased activity with aging, but

others have shown increases in complex II [20,30], and no change in complex I and III [30], and complex V [22,31] with aging. Importantly, all of these studies have utilized measurements of activity and function, without quantitation of protein levels, and have not identified gender differences. Actually one study, which examined both genders in skeletal muscle mitochondrial regulation with aging, found no gender differences [32].

Accordingly, the unique features of the current study include: (1) the novel primate model of aging, (2) the use of a proteomic approach in the heart, (3) identification of LV protein levels, (4) identification of specific proteins not noted to be altered in aging previously, and (5) marked gender differences. The vast majority of experimental studies on aging have utilized rodent models. Thus, the current investigation in aging is not only the first to utilize a proteomic approach in the heart, but also the first to utilize this approach in a primate model of aging. As noted above, in addition to the changes in activity and function of metabolic and mitochondrial pathways, we also observed downregulation of specific proteins, not observed previously in the heart, e.g. α-enolase, triosephosphate isomerase, pyruvate dehydrogenase E1 β-subunit, ATP-specific succinyl-CoA synthetase β-subunit, and 3-oxoacid CoA transferase.

A major finding of the current investigation was the marked gender difference in proteomic changes in the heart in aging monkeys. Most prior work failed to examine gender differences. The gender differences in the current investigation could be linked to our previous finding that there is a clear desensitization to β-adrenergic receptor stimulation in OM monkeys but preservation of β-adrenergic receptor responsiveness in OF monkeys [33]; further, the OM monkeys exhibited evidence of beginning aging cardiomyopathy not observed in OF monkeys. Adrenergic stimulation enhances the contribution of glucose to overall cardiac metabolism [34,35], and OM monkeys showed a gender-specific decreased utilization of glucose (glycolysis and glucose oxidation). Essentially, sympathetic stimulation of the heart through β-adrenergic receptor stimulation increases its utilization of glucose through four major mechanisms: increase in glucose uptake, increase in glycolysis, increase in glycogenolysis and increase in glucose oxidation. It is therefore possible that the decreased expression of glycolytic enzymes observed in aging males is related to the gender-specific β-adrenergic receptor desensitization [33], and that these two mechanisms are linked in aging males, which could act together to result in impaired cardiac function.

Since there was no evidence for atherosclerosis in the aorta of the monkeys examined by histopathology and no evidence for diabetes, as assessed by blood glucose levels, the downregulation of metabolic, and mitochondrial respiratory chain enzymes in aging males should be caused solely by aging. This is a key point, since this downregulation is also observed in patients with various forms of heart disease [36]. For example, the capacity of mitochondria for oxygen consumption and oxidative phosphorylation is significantly reduced in the failing heart [37–39]. Heart failure patients have decreased glucose uptake and enhanced fatty acid oxidation that may contribute to contractile dysfunction [39,40]. Mitochondrial respiratory chain complex III was decreased in patients with idiopathic dilated cardiomyopathy [41], and complex IV was decreased with ischemia [42]. In hearts of genetically diabetic mice with contractile dysfunction, the rates of glycolysis, and glucose oxidation were significantly lower than controls. In contrast, palmitate oxidation was increased 2-fold corresponding to the enhanced fatty acid oxidation seen in patients with heart failure [43]. In transgenic mice with cardiac-specific overexpression of PPARα, an increase in fatty acid utilization, and reduced glucose uptake mimics the metabolic and physiological abnormalities of the diabetic cardiomyopathic heart [44]. Therefore, it is conceivable that the metabolic alterations in aging males could eventually result in impaired cardiac function and explain in part the cardiomyopathy of aging, and conversely the absence of these changes could explain, in part, why aging females are protected longer than males, and live longer, independent of the delay in developing atherosclerosis following menopause.

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