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Research article

Metabolic changes during evolution of Sjögren's in both an animal model and human patients

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A B S T R A C T

Sjögren's (SS) involves salivary and lacrimal gland dysfunction. These studies examined metabolic profiles in the B6. *Il14α* transgene mouse model of SS and a cohort of human SS patients at different stages of disease. In B6. *Il14α* mice, products of glucose and fatty acid were common at 6 months of age, while products of amino acid metabolism were common at 12 months of age. Treating B6. *Il14α* mice with the glycolysis inhibitor 2-deoxyglucose from 6 to 10 months of age normalized salivary gland secretions, dacryoadenitis, hypergammaglobulinemia and physical performance, while treatment from 10 to 14 months of age failed to improve any of the clinical manifestations. Similarly, SS patients at an early stage of disease showed high glycolysis. SS patients with long-standing disease utilized predominantly amino acid metabolism, like B6. *Il14α* mice at 10–12 months of age. Additional studies are suggested to further define metabolic activities at the various disease stages.

1. Introduction

Sjögren's (SS) is an autoimmune disease with variable clinical symptoms manifest in patients that includes loss of salivary and/or lacrimal gland functions. Additional clinical manifestations can include lung disease, kidney disease, various neurological disorders, and a variety of malignancies, especially non-Hodgkin's B cell lymphomas [1]. Unfortunately, studies with patients continue to be hampered by a general delay in correct diagnoses of SS, which often takes several years, and limited access to relevant tissues [2,3]. To partially compensate, a variety of animal models, mostly of murine origin, have been developed that exhibit pathologies mimicking SS disease. These models have permitted in-depth analyses of individual pathological events as well as the timing of their onset and development [4,5]. The studies in this manuscript utilize the *Il14α* gene knock in (KI) B6. *Il14α* transgenic (TG) mouse developed in the laboratory of Dr. Julian Ambrus [6,7]. Findings from this animal models continue to be confirmed in SS patients [8,9].

While the majority of research evaluating the development of pathology in SS has focused on the activation of particular cell types and contribution of various cytokines, more recent studies are emphasizing how changes in metabolism influence the behavior of the immune system in autoimmunity [10–16]. Furthermore, preliminary studies in both SS patients and animal models have documented alterations in gene profiles in the salivary and lacrimal glands regulating corresponding metabolic pathways [17–20]. Interestingly, many SS patients with fatigue have been shown to have underlying metabolic disorders [21–24]. Treatment of the metabolic disorder leads to improvement in their fatigue [22,23,25,26]. Basic studies have demonstrated that effector cells generally require both aerobic

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and anaerobic glycolytic metabolism while regulatory cells rely on fatty acid metabolism [27–33]. We suspected that the nature of the metabolism would change as the disease evolved because different cells and cellular pathways were involved at different stages of the disease [8].

We initiated the current studies to better define how metabolism influences the development of SS and to determine which metabolic changes occur during the evolution of the disease. Three studies were undertaken [1]: general metabolomic studies in TG mice [2], treatment of TG mice at specific time points in their clinical manifestations with inhibitors of specific metabolic pathways to determine their effects on various disease parameters, and [3] a metabolomic study with SS patient-derived specimens utilizing patients with both recent onset and long-standing disease. With regards to the inhibitors of various metabolic pathways, we utilized metformin to block the mitochondrial respiratory chain [34], deoxy-glucose to specifically block all forms of glycolysis [35] and rapamycin to block mTOR that ultimately reduces glycolysis but also has other metabolic effects [36]. We hypothesized that different metabolic pathways would be used during the initial acute inflammatory stage of Sjogren's compared to the more chronic inflammatory phase that occurs as the disease is more established and different populations of cells are involved in the process.

2. Materials and methods

2.1. Mice

The IL14a transgenic mouse (TG) has been described as a model for SS [7,8]. Mice were bred and maintained in the animal facilities of the State University of New York (SUNY) at Buffalo and treated under the direction of the local IACUC committee. Six female mice were used for each treatment condition. Treatments consisted of deoxy glucose 800 mg/kg in saline given 5 times per week, metformin 100 mg/kg in saline given 5 times per week, or rapamycin 1 mg/kg in 0.2 % carboxymethylcellulose given 3 times per week. Control mice were given saline (for deoxy-glucose and metformin) or 0.2 % carboxymethylcellulose (for rapamycin). All injections were done intraperitoneally. Deoxy-glucose and metformin were purchased from Sigma-Aldrich (catalogue #D6134 and PHR1084, respectively), and rapamycin was purchased from LC Laboratories (Catalogue # R-5000). At the end of the treatment period, salivary flow was determined after pilocarpine stimulation, as previously described [7,8], and serum was isolated for metabolomic studies. Eye disease was determined by observation. The development of lymphomas was determined by histologic sectioning of the abnormal growths in the salivary glands and gastrointestinal tract. Wild type mice were female C57BL/6 mice used at the same ages as the TG mice. All procedures were approved by the SUNY IACUC under protocol MED 38116N. Animal studies complied with the ARRIVE guidelines in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals.

2.2. Treadmill testing

Treadmill tests were done, as described in detail elsewhere [37]. The mice were acclimatized to the treadmill device (Columbus instruments) one month prior to the test by giving three trials where the belt accelerated from 5 to 10 m/min over 5 mi. Two weeks prior to the test, the mice were given a single trial where the belt accelerated from 5 to 20 m/min over 30 min. The mice were finally tested by a single trial where the belt accelerated from 5 to 35 m/min over 60 min, and they were assessed for total time on belt (TOB) before exhaustion (defined as the mouse visiting the shock grid 10 times or receiving 20 total shocks).

2.3. Metabolomics

Metabolite profiling was performed by the Metabolite Profiling Core Facility at the Whitehead Institute (MIT), according to published protocol [38]. Polar metabolites were extracted from the serum as follows: 10 μl serum was mixed with 90 μl extraction solvent (75:25:0.2 acetonitrile:methanol:formic acid containing 909 nM each of 17 isotopically labeled amino acids, Cambridge Isotope Labs product number MSK-A2-1.2) in a standard Eppendorf tube, vortexed for 5 min at 4 °C and centrifuged at maximum speed

Table 1
Patients Studied.

Type	Age	Sex	Clinical Manifestations	Medications
Normal	55	female	none	none
Normal	75	male	hypertension	verapamil
Normal	50	male	none	none
Normal	35	male	none	none
Normal	33	male	none	none
Early SS	61	female	Dry eye, dry mouth, arthritis, Raynaud's	hydroxychloroquine, verapamil
Early SS	75	male	Dry eye, dry mouth, arthritis	methotrexate
Early SS	49	female	Dry eye, dry mouth, Raynaud's	verapamil
Early SS	33	male	Dry eye, dry mouth, arthritis	sulfasalazine
Early SS	79	male	Dry eyes, dry mouth, scleroderma	nifedipine
Late SS	63	female	Dry eye, dry mouth, peripheral neuropathy, Raynaud's	nifedipine
Late SS	75	female	Dry eye, dry mouth, Raynaud's	verapamil
Late SS	78	female	Dry eye, dry mouth, peripheral neuropathy	
Late SS	70	female	Dry eyes, dry mouth, polymyositis	IVIG

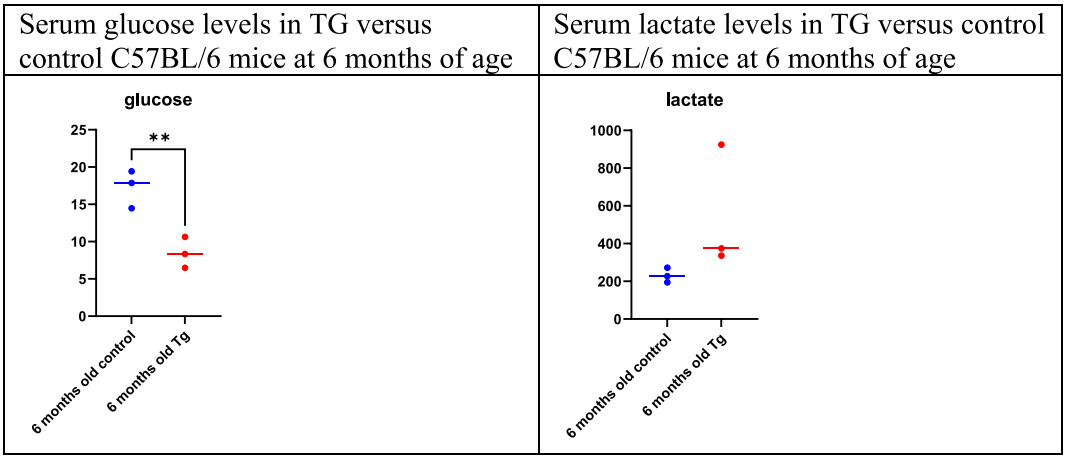


Fig. 1. Mass spectrometry of serum glucose and serum lactate levels in TG B6. Il14α mice versus control C57BL/6 mice at 6 months of age. Serum glucose (left panel) and serum lactate (right panel) were measured in sera of TG and C57BL/6 control mice using mass spectrometry. Each data point represents 3 mice at 6 months of age in each group. The differences in serum glucose levels between TG and control mice are statistically significant ($p = 0.0099$), but the difference in serum lactate values did not reach statistical significance ($p = 0.1766$). P-values were determined by unpaired *t*-test (two-tailed) using GraphPad Prism.

for 10 min at 4 °C. Supernatant was dried and resuspended in 100 μl of water.

Metabolite profiling was conducted on a QExactive bench top orbitrap mass spectrometer equipped with an Ion Max source and a HESI II probe, which was coupled to a Dionex UltiMate 3000 HPLC system (Thermo Fisher Scientific, San Jose, CA). External mass calibration was performed using the standard calibration mixture every 7 days. Typically, samples were reconstituted in 100 μL water and 2 μL were injected onto a SeQuant® ZIC®-pHILIC 150 × 2.1 mm analytical column equipped with a 2.1 × 20 mm guard column (both 5 mm particle size; EMD Millipore). Buffer A was 20 mM ammonium carbonate, 0.1 % ammonium hydroxide; Buffer B was acetonitrile. The column oven and autosampler tray were held at 25°C and 4°C, respectively. The chromatographic gradient was run at a flow rate of 0.150 mL/min as follows: 0–20 min: linear gradient from 80 to 20 % B; 20–20.5 min: linear gradient form 20–80 % B; 20.5–28 min: hold at 80 % B. The mass spectrometer was operated in full-scan, polarity-switching mode, with the spray voltage set to 3.0 kV, the heated capillary held at 275°C, and the HESI probe held at 350°C. The sheath gas flow was set to 40 units, the auxiliary gas flow was set to 15 units, and the sweep gas flow was set to 1 unit. MS data acquisition was performed in a range of $m/z = 70$–1000, with the resolution set at 70,000, the AGC target at $1x10^6$, and the maximum injection time at 20 msec. An additional scan (m/z 220–700) in negative mode only was included to enhance detection of nucleotides. Relative quantitation of polar metabolites was performed with XCalibur QuanBrowser 2.2 (Thermo Fisher Scientific) using a 5-ppm mass tolerance and referencing an in-house library of chemical standards. Further analysis of the data was done using MetaboAnalyst, an open-source R-based package.

2.4. Patients

Patients were recruited from the Immunology clinics at SUNY at Buffalo and the Ophthalmology clinics at the University of Pennsylvania. Normal controls were both age and sex matched friends of patients or independently recruited from SUNY at Buffalo staff and friends. The diagnosis of Sjogren's was based upon clinical symptoms, Schirmer's tests, measurement of saliva production and autoantibodies. All patients met the ACR/EULAR criteria for Sjogren's disease. Interestingly, of 23 recruited "normal", 10 had reduced tear and saliva production consistent with Sjogren's. Ultimately the studies included 5 normal (4 males and 1 female), 5 patients with early Sjogren's (<2 years; 3 males and 2 females) and 4 patients with late Sjogren's (>5 years; all female), who could be clearly assigned to one of the 3 groups without ambiguity. Patient recruitment and participation were approved under IRB protocol CR00003632. The work described was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human and patient consent.

The clinical features of the study subjects are outlined in Table 1. None of the patients had other major medical conditions.

This table list the individuals that were included in this study. There were 5 normal controls, 5 patients with early Sjogren's (<2 years; Early SS) and 4 patients with Sjogren's > 5 years (Late SS).

2.5. Statistical analysis

Statistical analysis of mouse treatment data was done by unpaired *t*-test (two-tailed) using GraphPad Prism. Analysis of mouse metabolomics data was done using MetaboAnalyst, an open-source R-based package. Analyses of patient data were carried out using ROC statistically determined Area under the Curve (AUC) that provides a probability threshold that ranks random samples. An ROC (Receiver Operating Characteristics) curve is a plot of true positive rate on the *y* axis against false positive rate on the *x* axis [39]. AUC (Area Under the Curve) of an ROC curve gives an estimate of diagnostic accuracy. AUC of 0.5 denotes random chance and AUC of 1

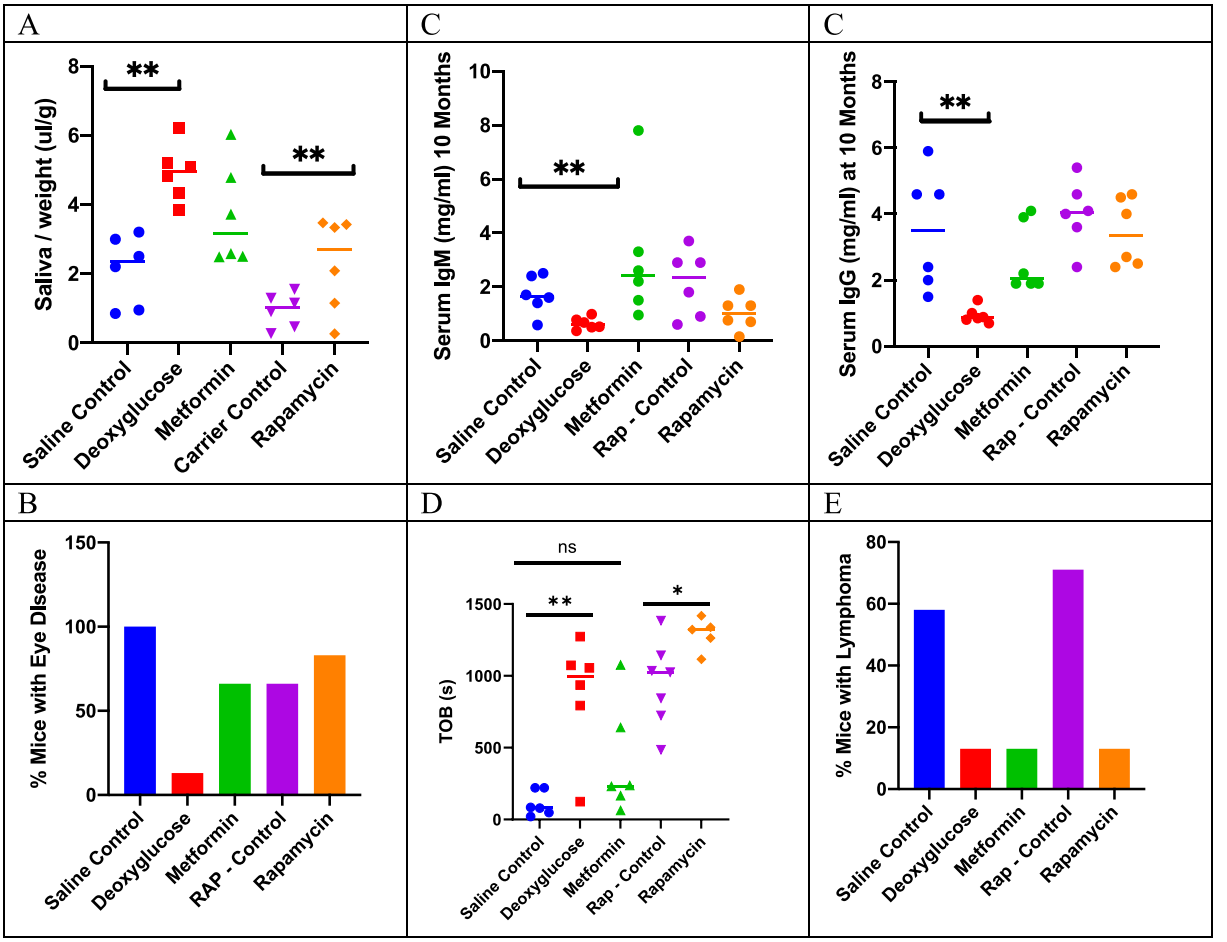


Fig. 2. Metabolic Pathways Inhibitor treatments at a relatively young age (6–10 months) differentially affect development and onset of SS in TG B6. Il14α Mice. A: Changes in Production of Saliva. Groups of 6 female IL14a Transgenic (TG) mice were treated with either deoxy glucose (800 mg/kg), metformin (100 mg/kg), rapamycin (1 mg/kg), saline or carrier control (for rapamycin) from 6 to 10 months of age. Saliva was collected at 10 months of age after pilocarpine injection. The difference between deoxy glucose and saline was statistically significant ($p = 0.004$) as was the difference between rapamycin and carrier control ($p = 0.04$). The difference between metformin and saline was not statistically significant ($p = 0.056$). This suggests that glycolysis is critical for the early salivary gland injury in this model. B: Same experiment as 3A. Only deoxy glucose prevented early eye injury, as determined by observation. C: Same experiment as 3A. Hypergammaglobulinemia, both IgM ($p = 0.0055$) and IgG ($p = 0.0056$) were significantly reduced in deoxy glucose mice compared to saline control mice. Differences in metformin treated mice were not statistically different compared to saline treated mice (IgM – $p = 0.222$, IgG = 0.335). The differences between rapamycin and rapamycin control treated mice were also not statistically significant (IgM – $p = 0.075$; IgG – $p = 0.351$). D: IL14 TG mice treated with deoxy glucose or Rapamycin from 6 to 10 months showed improved physical performance (when tested on a treadmill) compared to the mice treated with vehicle controls ($p = 0.0010$ for deoxy glucose, $p = 0.0339$ for Rapamycin). Metformin treatment did not give statistically significant results ($p = 0.1019$). E: This study looks at the development of Sjogren's related lymphoma in IL-14aTG mice (6 in each group) at 16 months of age after treatment with the listed agents from 8 to 14 months of age. Incidence of lymphoma was decreased by blocking glycolysis (deoxy glucose), metformin (inhibits mitochondrial respiratory chain) or rapamycin (block mTORC 1 thus blocking glycolysis and encouraging fatty acid oxidation). P-values were determined by unpaired *t*-test (two-tailed) using GraphPad Prism.

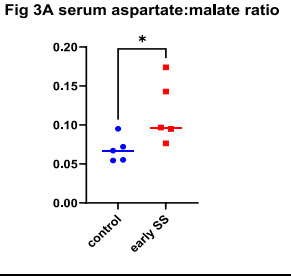
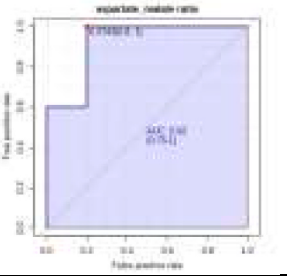
denotes perfect accuracy.

3. Results

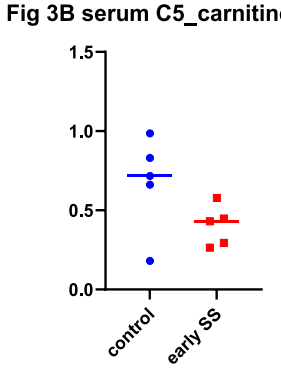
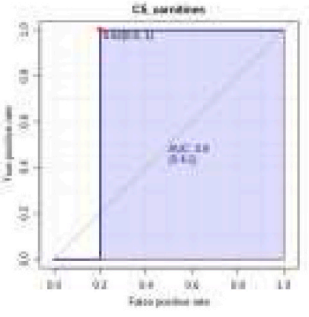
1. Alterations in serum metabolites of TG mice during the evolution of disease.

TG B6.*Il14α* mice exhibit many features of human SS observed in patients, including infiltration of the submandibular, lacrimal glands and lung tissues, loss of salivary gland secretions, upregulated type 1 interferons, and develop of B cell lymphomas [7,8]. In addition, SS disease onset in these mice occurs in the same relative time as in humans. However, in-depth studies of metabolism in these mice are lacking; thus, we carried out a metabolomic examination of TG sera collected at 6 and 12 months of age. Metabolic

A

Serum Aspartate: Malate Ratio	ROC AUC
	

B

Serum C5 Carnitine Level	ROC AUC
	

C

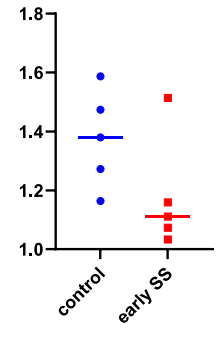
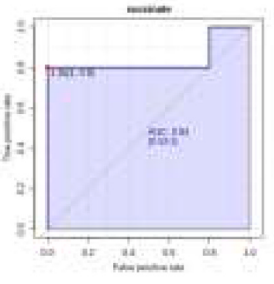
Serum Succinate Level	ROC AUC
	

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Fig. 3. Serum Markers Distinguishing Patients with Early Sjogren's Syndrome from Normal Controls.A. Serum aspartate and malate were measured in the sera of patients with early SS and normal controls by mass spectrometry as described in Materials and Methods. Data shown are shown for 5 individuals in each group. The difference in aspartate – malate ratio between early SS and normal controls was statistically significant ($p = 0.038$). The ROC graph documents the validity of the biomarker, based on its high sensitivity, specificity and area under the curve (AUC). B. Serum C5-carnitine was measured in the sera of patients with early SS and normal controls by mass spectrometry as described in Materials and Methods. Data shown are shown for 5 individuals in each group. The difference in C5 carnitine between patients with early SS and normal controls were not statistically significant ($p = 0.10137$). The ROC graph documents the validity of the biomarker, based on its high sensitivity, specificity, and area under the curve. (AUC). C. Serum succinate was measured in the sera of patients with early SS and normal controls by mass spectrometry as described in Materials and Methods. Data shown are shown for 5 individuals in each group. Even though the difference in succinate between patients with early SS and normal controls was not statistically significant ($p = 0.121$), this can be a useful marker for a group of patients with SS, since the low values are only given by patients with SS. The ROC graph documents the validity of the biomarker, based on its high sensitivity, specificity and area under the curve (AUC). P-values were determined by unpaired *t*-test (two-tailed) using GraphPad Prism.

profiles of these two time points are presented in Supplemental Fig. 1 and reveal quite different overall metabolic activities between SS-susceptible and SS-non-susceptible mice. Furthermore, as presented in Fig. 1, a direct comparison of serum glucose versus serum lactate in these two strains indicates that glucose levels *per se* are decreased in TG mice relative to control mice at 6 months of age with a corresponding elevation in lactate in TG mice compared to control mice. This altered metabolism correlates with the timing of SS pathology onset in TG mice.

2. Clinical response to therapies targeting different metabolic pathways in TG mice.

To better understand the significance of these findings, TG mice were treated long-term with three metabolic inhibitors, i.e., 2-deoxyglucose (DG) [40], which inhibits glycolysis, metformin (M), which inhibits the activity of the mitochondrial respiratory chain [34] or rapamycin (RP), which blocks mTOR [41]. As presented in Fig. 2, mice treated for 4 weeks with DG starting at 6 months of age showed normalization of salivary gland secretions, eye disease, hypergammaglobulinemia, and physical performance. Mice treated with RP had modest improvement in salivary gland secretions, no change in eye disease, reduced IgM, but no changes in IgG. In contrast, M treatment did not improve any of these clinical manifestations. DG completely blocks glycolysis while RP reduces the reliance on glycolysis by blocking mTOR. In total contrast, TG mice treated with DG, M or RP from 10 to 14 months of age exhibited no significant changes in disease manifestations, supporting the fact that at this later timepoint the primary metabolic activity does not involve glycolysis. Amino acid metabolism is critical at this stage (Supplemental Fig. 1).

3. Serum Metabolites in Patients with Recent Onset or Long-Standing SS

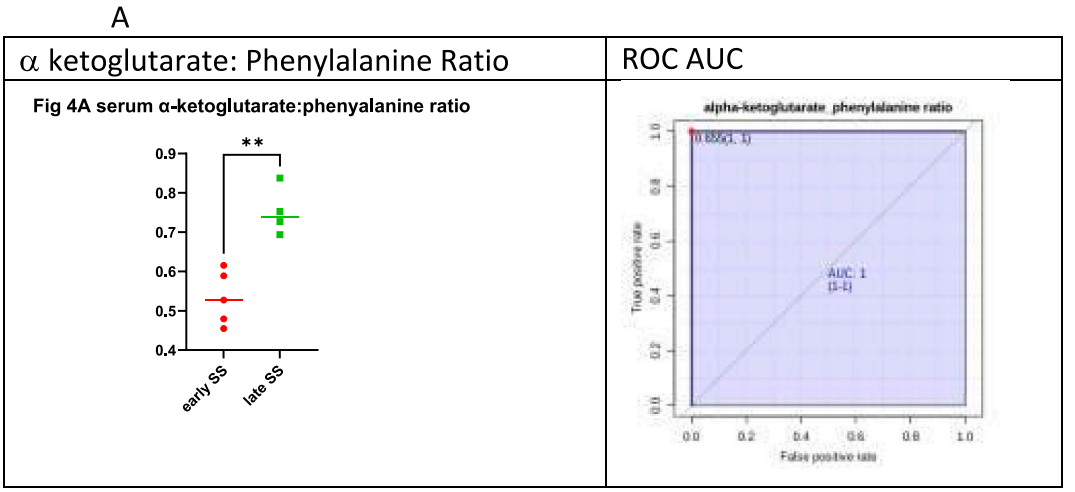
Based on these metabolic data obtained from our TG B6. *Il14α* mouse model, we next asked whether human SS patients show similar age-dependent metabolic profiles. For this study, we recruited two groups of SS patients, those with a disease diagnosis less than 2 years and those with disease more than 5 years. A non-disease control group of participants were friends of the patients; however, many of these "normal control" participants were individuals interested to know if they might have early-stage SS, and in fact, several were determined to have decreased salivary and lacrimal gland secretions. As a result, and together with clinical histories, Schirmer's tests, salivary gland secretions and autoantibodies, we were able to place the test individuals into one of 3 categories: normal controls, early-stage SS or late-stage SS. These studies are based on 5 normal controls, 5 early-stage SS patients, and 4 long-term SS patients, who could be clearly assigned to one of the 3 groups without ambiguity.

Metabolic profiling carried out with the participating individuals assigned to one of the 3 groups indicate similar profiles as observed in our murine studies above, i.e., patients with early-stage SS have different metabolic profiles compared to long-standing disease and both disease groups metabolic profiles are different than those of the normal controls. The pathways most prevalent in the patients with early disease include utilization of the mitochondrial respiratory chain, utilization of ketone bodies and utilization of the malate: aspartate shuttle, implying glycolysis, but a possible insufficient source of glucose. The pathways most prevalent in patients with long-standing disease indicate amino acid metabolism, like what was seen in TG mice. Interestingly, differences in the metabolic profiles between the patients with early disease and those with long-standing disease were not as dramatic as the differences between the TG mice at 6 versus 12 months of age, likely reflecting the greater genetic diversity in SS patients *per se* as well as genetic difference between humans and in-bred mice (Supplemental Fig. 2).

Based on these observations, it is not surprising that serum markers that distinguish normal control participants from both patients with either early- or late-stage SS include a high aspartate: malate ratio (Fig. 3A) and a low concentration of C5carnitine (Fig. 3B) or a low concentration of succinate (Fig. 3C). Serum markers that distinguish patients with early SS from those with long-standing SS include a low α-ketoglutarate: phenylalanine ratio (Fig. 4A) and a low glucose: valine ratio (Fig. 4B). Serum markers that distinguish patients with long-standing SS from normal controls include adenosine, histidine and the adenosine: inosine ratio (Fig. 5A–C). These data suggest the use of DNA and amino acid metabolism in patients with long-standing SS.

4. Discussion

Metabolism is an essential function of every cell so it is not surprising that changes in metabolism are noted in an autoimmune disease where tissue damage occurs secondary to abnormal cellular function [19,20,24,42–47]. A growing literature documents



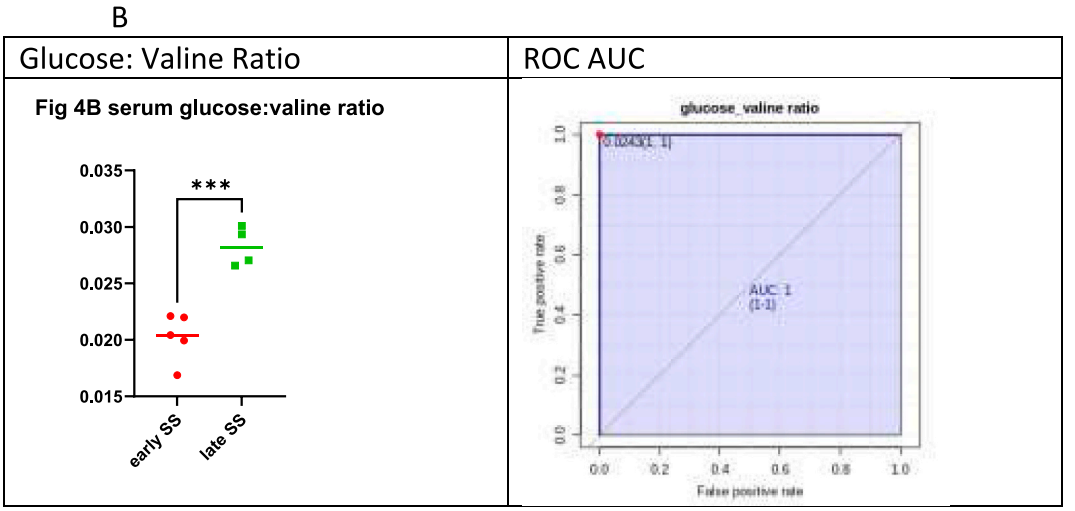
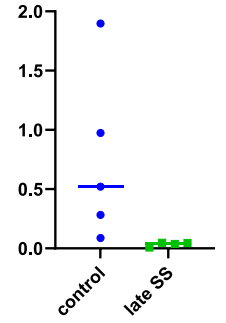
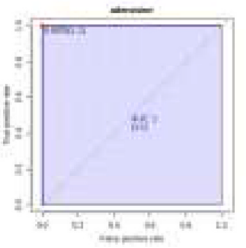


Fig. 4. Serum Markers Distinguishing Patients with Early Sjogren's Syndrome from Patients with Long Standing Sjogren's Syndrome. A. Serum alpha ketoglutarate and phenylalanine were measured in the sera of patients with early SS and long-standing SS by mass spectrometry as described in Materials and Methods. Data shown are shown for 5 individuals with early SS and 4 individuals with long-standing SS. The difference in alpha ketoglutarate to phenylalanine ratio between patients with early SS and patients with long -standing SS was statistically significant ($p = 0.002$). The ROC graph documents the validity of the biomarker, based on its 100 % sensitivity, specificity and area under the curve (AUC). B. Serum glucose and valine were measured in the sera of patients with early SS and long-standing SS by mass spectrometry as described in Materials and Methods. Data shown are for 5 individuals with early SS and 4 individuals with long-standing SS. The difference in the glucose: valine ratio between patients with early SS and patients with long-standing SS was statistically significant ($p = 0.0005$). The ROC graph documents the validity of the biomarker, based on its 100 % sensitivity, specificity and area under the curve (AUC). P-values were determined by unpaired *t*-test (two-tailed) using GraphPad Prism.

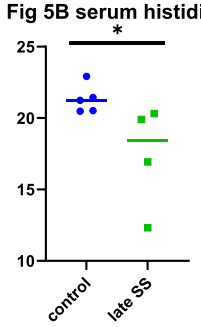
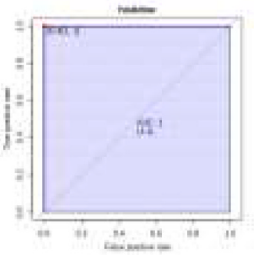
metabolic abnormalities in SLE [48–51]. In the current study, we examined whole body metabolism by evaluating metabolites in sera. Whole body metabolism reflects predominantly changes occurring in the muscle, liver and brain, but may also reflect large changes occurring in other dominant cell types and tissues [43,52]. When cells go from a resting to an activated state, changes in metabolism are required to allow phenotypic changes to occur [53]. Resting B and T lymphocytes go from using predominantly oxidative phosphorylation to aerobic and anaerobic glycolysis for rapid generation of ATP as they become effector cells. Regulatory T cells rely more on fatty acid metabolism. TH17 cells utilize amino acid metabolism along with glycolysis [16]. At the same time, however, the substrates generated from whole body metabolism, limit and may dictate the nature of cellular differentiation and effector function that may occur [30,43,44,52,54–57].

The first set of studies documented in the TG mouse that glycolytic metabolism was prevalent in the early stages of the disease, while fatty acid and amino acid metabolism was prevalent in the later stages of the disease. Since effector cells generally use glycolytic

A

Adenosine	ROC
Fig 5A serum adenosine 	

B

Histidine	ROC
Fig 5B serum histidine 	

C

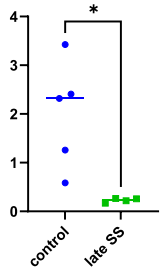
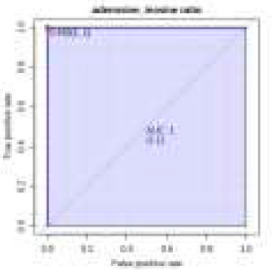
Adenosine: Inosine Ration	ROC
Fig 5C serum adenosine:inosine ratio 	

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Fig. 5. Distinguishing Patients with Long-Standing Sjogren's Syndrome from Normal Controls. A. Serum adenosine was measured in the sera of patients with long-standing SS and normal controls by mass spectrometry as described in Materials and Methods. Data shown are shown for 5 controls and 4 patients. The difference in adenosine between patients with long-standing SS and normal controls was not quite statistically significant ($p = 0.09$). The ROC graph documents the validity of the biomarker, based on its 100 % sensitivity, specificity and AUC. B. Serum histidine was measured in the sera of patients with longstanding SS and normal controls by mass spectrometry as described in Materials and Methods. Data shown are for 5 controls and 4 patients. The difference in histidine between patients with long-standing SS and normal controls was statistically significant ($p = 0.053$). The ROC graph documents the validity of the biomarker, based on 100 % sensitivity, specificity, and AUC. C. Serum adenosine and inosine were measured in the sera of patients with long-standing SS and normal controls by mass spectrometry as described in Materials and Methods. Data are shown for 5 controls and 4 patients. The difference in adenosine: inosine ratio between patients with long-standing SS and normal controls was statistically significant ($p = 0.016$). The ROC AUC graph documents the validity of the biomarker, based on its 100 % sensitivity, specificity, and AUC. P-values were determined by unpaired *t*-test (two-tailed) using GraphPad Prism.

metabolism, it suggests that early in the course of the disease cells are activated to induce tissue damage leading to salivary and lacrimal gland dysfunction. The challenge has been to understand which cells are activated and how does that loss of salivary and lacrimal gland dysfunction occur. Previous work by Blokland et al. documented activation of mTORC1 in B and T cells from patients with Sjogren's syndrome [58]. Work by Perl et al. documented activation of mTORC1 in SLE by kynurenine [59] and this has been confirmed by Bengtsson et. AL. in SLE and SS [60]. We did not evaluate the mechanisms leading to glycolysis in these studies. Work in the TG model as well as in other animal models for SS have suggested that loss of salivary gland function occurs before lymphocyte infiltration is identified in the salivary glands [1,4,61]. Marginal zone B cells (MZB) have been shown to be critical for secretory gland damage, as has lymphotoxin (LT), which can be produced by MZB [7,62–68]. Further studies are needed to specifically analyze the metabolism in MZB in SS models and patients, although studies from several laboratories have identified glycolytic metabolism in activated B cells [11,69–71]. Furthermore, another key question is whether metabolic abnormalities induced by some environmental insult cause the dysfunction of the MZB cells, or whether a primary immunological process is reflected in the changes in metabolism that are identified. Also, is LT a direct mediator of secretory gland injury or does it work via indirect mechanisms, such as inducing local germinal center formation [72–74]. Does LT work via metabolic changes that it induces or are the metabolic changes causing LT secretion and local cellular dysfunction [75–77].

The second set of studies demonstrates that blocking glycolysis does in fact prevent the physiological abnormalities identified in early SS in the TG mouse. It also confirms that in later stages of SS, blocking glycolysis is not effective. The nature of metabolism in later stages of SS is very different from that in early stages of SS, involving predominantly amino acid metabolism. It suggests that treatment of patients with SS requires evaluation of the nature and stage of their disease [8]. In studies with patients with SLE, blocking mTOR with Sirolimus did lead to improvements in the SLEDAI score [78]. Interestingly, rapamycin blocks mTORC1 more effectively than mTORC2 while deoxy glucose blocks all types of glycolysis [79]. In our studies deoxy glucose was more effective than rapamycin in blocking all clinical manifestations that were evaluated.

The third set of studies demonstrates that patients with SS behave in a very similar, but not identical manner, to TG mice. This is not surprising as TG mice are genetically homogeneous living in a homogenous environment and patients with SS are genetically heterogenous living in very different and changeable environments. Patients with early SS utilized the mitochondrial respiratory chain and glycolysis, as in TG mice. Patients with early SS, in contrast to TG mice, utilize ketone bodies [80] and the malate – aspartate shuttle [81] to generate necessary energy as there is not sufficient glucose for glycolysis alone to provide the needed energy. Interestingly, succinate, an intermediary in both the citric acid cycle and the mitochondrial respiratory chain, which can have a number of activities contributing to inflammation, is decreased in patients with early SS compared to normal controls [82–85]. Perhaps this is an attempt of the body to reduce inflammation stemming from other sources. In patients with long – standing SS as in TG mice with long – standing disease, amino acid metabolism is a main source for energy generation. Interestingly, previous studies had documented increased activation of mTORC1 in B and T cells from patients with Sjogren's, supporting our findings of increased glycolysis; these studies did not characterized the stage of disease of the patients [58].

Overall, these studies demonstrate that the evolution of SS in TG mice and patients involves changes in metabolism. The strength of these studies is the work in the animal model in which the significance of the observed metabolic changes could be confirmed using in vivo metabolic inhibitors. Further studies are necessary to dissect out the mechanisms involved in inducing these metabolic changes. The weakness of these studies is the number of subjects in the human studies. This was partly due to strict requirements for placing subjects in the 3 categories: Early SS, Late SS and normal controls. The normal controls were not age and sex matched to the study subjects. Further studies are needed with larger numbers of subjects to confirm the findings in this study. Nonetheless, the human studies did confirm the validity of the murine studies to help direct further research. Additional studies are needed to further characterize the metabolic changes observed in these studies and evaluate whether they either dictate changes in cellular function and/or are the results of changes in cellular composition. The identification of these changes may ultimately help with the identification of patients with SS at an early stage of disease, when treatment can reverse tissue damage. Changes in metabolism may occur before changes in particular immunological parameters, such as cytokine and autoantibodies. Changes in metabolism may be responsible for inducing changes in production of cytokines and autoantibodies. New treatment strategies may ultimately involve preventing or blocking the metabolic changes leading to all the subsequent downstream immunological and biological events.

CRediT authorship contribution statement

Alexander Jacob: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Jing He:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Ammon Peck:** Writing – review & editing, Formal analysis, Conceptualization. **Ali Jamil:** Investigation, Data curation. **Vatinee Bunya:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Jessy J. Alexander:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Julian L. Ambrus:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

Ethics declaration

This work and this publication were performed according to Elsevier's Publishing Ethics Policy.

This work has not been previously published. The publication was approved by the authors. None of the authors have conflicting interests. AI was not utilized in any aspect of this publication. For animal studies, all procedures were approved by the SUNY IACUC under protocol MED 38116N. Animal studies complied with the ARRIVE guidelines in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals. For patient studies, patient recruitment and participation were approved under IRB protocol CR00003632. The work described was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving human and patient consent.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Julian Ambrus reports was provided by University at Buffalo Jacobs School of Medicine and Biomedical Sciences. Julian L. Ambrus Jr., MD reports a relationship with Amgen Inc that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e41082.

Data availability

Data is available on request from the Ambrus lab. Contact Julian L. Ambrus Jr., MD at jambrus@buffalo.edu. The metabolomics data are available from the Harvard Dataverse: https://doi.org/10.7910/DVN/J2XYRN Clickto follow link.">https://doi.org/10.7910/DVN/J2XYRN.

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