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Mechanotransduction in Ischemic Cardiac Tissue: A Mechanical Bidomain Approach under Plane Stress

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ABSTRACT
Mechanotransduction is the process by which biological tissue translates mechanical forces and signals, such as those produced by strains or membrane forces, into biological reactions including cell remodeling, growth, and differentiation. While some analyses assume strain (the derivative of either the intracellular or extracellular displacement) as the cause of mechanotransduction, this paper assumes that differences between the intracellular and extracellular displacements, known as membrane force, result in mechanical forces acting on integrin proteins, causing mechanotransduction. The mechanical bidomain model is a two-dimensional mathematical representation that describes this behavior. Previous analyses describe mechanotransduction using plane strain, which assumes zero displacement in the z-direction. This analysis uses plane stress, which assumes zero stress in the z-direction, to describe where mechanotransduction occurs in comparison to plane strain models. A sample of healthy tissue with a circular ischemic region with no active tension in the center is analyzed using numerical methods. Fixed and free boundary conditions are implemented. Under fixed conditions, the membrane force was largest in the ischemic border zone and zero everywhere else. However, the strain was found to be largest in the ischemic region. Under free conditions, the membrane force was largest on the vertical edges and in the ischemic border zone. The strain was found to be nearly zero in the ischemic region and ranged up to 10% throughout the tissue. In conclusion, this paper found that both plane strain and plane stress predict a membrane force in the ischemic border zone, but the distribution of individual displacements and strain vary according to each model. These results are significant in determining which model is most appropriate to use in predicting how mechanical forces affect cellular remodeling when analyzing thin monolayers of tissue.

KEYWORDS
Mechanotransduction; Plane Stress; Tissue Engineering; Mechanical Bidomain Model; Biomechanics; Strain; Intracellular Cytoskeleton; Extracellular Matrix; Ischemia; Finite Differences

INTRODUCTION
When cardiac tissue is subjected to a force, the individual cells react and change via a process called mechanotransduction, which is a cell's biological response to a mechanical stimulus. More specifically, integrin proteins, connecting the intracellular cytoskeleton and the extracellular matrix, are predicted to be the components that induce mechanotransduction when a force causes a difference in displacement between the intra- and extracellular spaces.[1] In effect, the growth, remodeling, and differentiation of the cells have been hypothesized as being the results of mechanotransduction.[2] To predict and describe this behavior, a mathematical model called the mechanical bidomain model has been developed.[3] This model is macroscopic in that it relates the interactions between the intracellular and extracellular spaces separately through forces on integrin membrane proteins (the membrane force).[3,4] The model also predicts the stress (force per unit area) and strain (change in length divided by the original length) existing throughout both the cytoskeleton and the extracellular matrix.

In previous models, the two-dimensional case of plane strain was analyzed; it assumed no tissue displacements perpendicular (z-direction) to the tissue.[3] However, numerous experiments are conducted on thin monolayers of cells that can experience displacement in all directions. For the case of plane stress, in comparison, displacements can occur in the z-direction, but no stress exists in this direction because there is no force acting normal to the cell monolayers.[5] This analysis examines a rectangular sheet of tissue with a circular ischemic (oxygen deprived) region in the center, similar to that examined by Gandhi and Roth for plane strain.[6] The purpose of this paper is to analyze mechanotransduction under plane stress and compare the results to the previous analysis under plane strain to determine possible implications of the effects of mechanotransduction in cardiac tissue. Our mathematical simulations of mechanotransduction around a region of ischemia may provide insight into how cardiac tissue remodels following a heart attack.

METHODS AND PROCEDURES

The mechanical bidomain model was used to describe the behavior of the mechanical force, in the form of stresses and strains, acting upon a sheet of cardiac tissue. This model was used to derive equations with plane stress, which assumes that the tissue has zero stress in the z-direction and is incompressible (no change in volume). Stress (τ) and strain (ε) are related by

$$\tau_{ixx} = -p + 2v\varepsilon_{ixx} + T \qquad \tau_{iyy} = -p + 2v\varepsilon_{iyy} \qquad \tau_{izz} = -p + 2v\varepsilon_{izz}$$

$$\tau_{ixy} = 2v\varepsilon_{ixy} \qquad\qquad \tau_{ixz} = 2v\varepsilon_{ixz} \qquad\qquad \tau_{iyz} = 2v\varepsilon_{iyz}, \qquad\qquad \textbf{Equation 1.}$$

where p is the intracellular hydrostatic pressure, v is the intracellular shear modulus relating the strain to the stress, and T is the active tension produced by actin and myosin molecules that act in the direction along the fibers (*x*-direction). The intracellular strains are defined with respect to the intracellular tissue displacement, **u**, by

$$\varepsilon_{ixx} = \frac{\partial u_x}{\partial x} \qquad\qquad \varepsilon_{iyy} = \frac{\partial u_y}{\partial y} \qquad\qquad \varepsilon_{izz} = \frac{\partial u_z}{\partial z}$$

$$\varepsilon_{ixy} = \frac{1}{2}\left(\frac{\partial u_x}{\partial y} + \frac{\partial u_y}{\partial x}\right) \qquad \varepsilon_{ixz} = \frac{1}{2}\left(\frac{\partial u_x}{\partial z} + \frac{\partial u_z}{\partial x}\right) \qquad \varepsilon_{iyz} = \frac{1}{2}\left(\frac{\partial u_y}{\partial z} + \frac{\partial u_z}{\partial y}\right). \qquad \textbf{Equation 2.}$$

Similar equations exist for the extracellular space,

$$\tau_{exx} = -q + 2\mu\varepsilon_{exx} \qquad \tau_{eyy} = -q + 2\mu\varepsilon_{eyy} \qquad \tau_{ezz} = -q + 2\mu\varepsilon_{ezz}$$

$$\tau_{exy} = 2\mu\varepsilon_{exy} \qquad\qquad \tau_{exz} = 2\mu\varepsilon_{exz} \qquad\qquad \tau_{eyz} = 2\mu\varepsilon_{eyz}, \qquad\qquad \textbf{Equation 3.}$$

and

$$\varepsilon_{exx} = \frac{\partial w_x}{\partial x} \qquad\qquad \varepsilon_{eyy} = \frac{\partial w_y}{\partial y} \qquad\qquad \varepsilon_{ezz} = \frac{\partial w_z}{\partial z}$$

$$\varepsilon_{exy} = \frac{1}{2}\left(\frac{\partial w_x}{\partial y} + \frac{\partial w_y}{\partial x}\right) \qquad \varepsilon_{exz} = \frac{1}{2}\left(\frac{\partial w_x}{\partial z} + \frac{\partial w_z}{\partial x}\right) \qquad \varepsilon_{eyz} = \frac{1}{2}\left(\frac{\partial w_y}{\partial z} + \frac{\partial w_z}{\partial y}\right), \qquad \textbf{Equation 4.}$$

where **w** is the extracellular displacement, q is the extracellular hydrostatic pressure, and μ is the extracellular shear modulus accounting for the elastic properties of the extracellular matrix. Incompressibility implies that

$$\varepsilon_{ixx} + \varepsilon_{iyy} + \varepsilon_{izz} = 0 \qquad\qquad\qquad \textbf{Equation 5.}$$

$$\varepsilon_{exx} + \varepsilon_{eyy} + \varepsilon_{ezz} = 0. \qquad\qquad\qquad \textbf{Equation 6.}$$

The incompressibility condition indicates that a shortening in one direction must be accompanied by a lengthening in another direction. Since plane stress means the stresses normal to the tissue are zero, $\tau_{izz} = \tau_{ixz} = \tau_{iyz} = \tau_{ezz} = \tau_{exz} = \tau_{eyz} = 0$. One implication of plane stress is that $p = -2v(\varepsilon_{ixx} + \varepsilon_{iyy})$ and $q = -2\mu(\varepsilon_{exx} + \varepsilon_{eyy})$. Plugging q and p into **Equation 1** and **Equation 3**, the intra- and extracellular stresses become

$$\tau_{ixx} = 2v\left[2\frac{\partial u_x}{\partial x} + \frac{\partial u_y}{\partial y}\right] + T \qquad \tau_{iyy} = 2v\left[2\frac{\partial u_y}{\partial y} + \frac{\partial u_x}{\partial x}\right] \qquad \tau_{ixy} = v\left[\frac{\partial u_x}{\partial y} + \frac{\partial u_y}{\partial x}\right] \qquad \textbf{Equation 7.}$$

$$\tau_{exx} = 2\mu\left[2\frac{\partial w_x}{\partial x} + \frac{\partial w_y}{\partial y}\right] \qquad \tau_{eyy} = 2\mu\left[2\frac{\partial w_y}{\partial y} + \frac{\partial w_x}{\partial x}\right] \qquad \tau_{exy} = \mu\left[\frac{\partial w_x}{\partial y} + \frac{\partial w_y}{\partial x}\right]. \qquad \textbf{Equation 8.}$$

The equations of static equilibrium are

$$\frac{\partial \tau_{ixx}}{\partial x} + \frac{\partial \tau_{ixy}}{\partial y} = K(u_x - w_x) \qquad\qquad \textbf{Equation 9.}$$

$$\frac{\partial \tau_{iyy}}{\partial y} + \frac{\partial \tau_{ixy}}{\partial x} = K(u_y - w_y) \qquad\qquad \textbf{Equation 10.}$$

$$\frac{\partial \tau_{exx}}{\partial x} + \frac{\partial \tau_{exy}}{\partial y} = -K(u_x - w_x) \qquad\qquad \textbf{Equation 11.}$$

$$\frac{\partial \tau_{eyy}}{\partial y} + \frac{\partial \tau_{exy}}{\partial x} = -K(u_y - w_y), \qquad\qquad \textbf{Equation 12.}$$

where K is a Hookean spring constant used to account for the elasticity of the integrins coupling the intra- and extracellular spaces.[3] The active tension (T) is assumed to be uniform throughout the tissue excluding the circular ischemic region defined by $R = \sqrt{x^2 + y^2} < a$ where $T=0$. We chose this geometry and fiber orientation because it is identical to the one analyzed by Gandhi and Roth.[6]

The boundary conditions at the outer edge of the tissue were either fixed or free. Initially, the boundaries were fixed such that the displacements **u = w** = 0, as was analyzed by Gandhi and Roth.[6] Alternatively, the boundaries were also modeled as being free such that the stress normal to the boundary is zero, as was analyzed by Sharma.[7]

Differentiating **Equation 7** and **Equation 8** and substituting them into **Equations 9-12** yields

$$v\left[4\frac{\partial^2 u_x}{\partial x^2} + 3\frac{\partial^2 u_y}{\partial x \partial y} + \frac{\partial^2 u_x}{\partial y^2}\right] + \frac{dT}{dx} = K(u_x - w_x) \qquad\qquad \textbf{Equation 13.}$$

$$v\left[4\frac{\partial^2 u_y}{\partial y^2} + 3\frac{\partial^2 u_x}{\partial x \partial y} + \frac{\partial^2 u_y}{\partial x^2}\right] = K(u_y - w_y) \qquad\qquad \textbf{Equation 14.}$$

$$\mu\left[4\frac{\partial^2 w_x}{\partial x^2} + 3\frac{\partial^2 w_y}{\partial x \partial y} + \frac{\partial^2 w_x}{\partial y^2}\right] = -K(u_x - w_x) \qquad\qquad \textbf{Equation 15.}$$

$$\mu\left[4\frac{\partial^2 w_y}{\partial y^2} + 3\frac{\partial^2 w_x}{\partial x \partial y} + \frac{\partial^2 w_y}{\partial x^2}\right] = -K(u_y - w_y). \qquad\qquad \textbf{Equation 16.}$$

To solve **Equations 13-16** numerically, the derivatives are replaced by finite differences and solved iteratively using overrelaxation.[6] The tissue sheet is described as a grid of points with $i=1,2,\ldots,N_x$ in the x-direction and $j=1,2,\ldots,N_y$ in the y-direction. Solving **Equations 13-16** for $u_x(i,j)$, $u_y(i,j)$, $w_x(i,j)$, and $w_y(i,j)$ in terms of their nearest neighbors, we obtained:

$$u_x(i,j) = \frac{\lambda\omega_x\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}\right)+\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}+10\lambda\right)\left(\theta_x+\frac{\Delta T_x}{2\nu}\right)}{\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}+10\lambda\right)\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}+10\right)-\left(\frac{\Delta^4\lambda^2}{\sigma^4(1+\lambda)^2}\right)} \qquad \textbf{Equation 17.}$$

$$w_x(i,j) = \frac{\lambda\omega_x\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}+10\right)+\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}\right)\left(\theta_x+\frac{\Delta T_x}{2\nu}\right)}{\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}+10\lambda\right)\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}+10\right)-\left(\frac{\Delta^4\lambda^2}{\sigma^4(1+\lambda)^2}\right)} \qquad \textbf{Equation 18.}$$

$$u_y(i,j) = \frac{\lambda\omega_y\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}\right)+\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}+10\lambda\right)\theta_y}{\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}+10\lambda\right)\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}+10\right)-\left(\frac{\Delta^4\lambda^2}{\sigma^4(1+\lambda)^2}\right)} \qquad \textbf{Equation 19.}$$

$$w_y(i,j) = \frac{\lambda\omega_y\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}+10\right)+\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}\right)\theta_y}{\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}+10\lambda\right)\left(\frac{\Delta^2\lambda}{\sigma^2(1+\lambda)}+10\right)-\left(\frac{\Delta^4\lambda^2}{\sigma^4(1+\lambda)^2}\right)} \qquad \textbf{Equation 20.}$$

where,

$$\theta_x = 4[u_x(i+1,j)+u_x(i-1,j)]+\frac{3}{4}[u_y(i+1,j+1)+u_y(i-1,j-1)-u_y(i-1,j+1)-u_y(i+1,j-1)]+$$
$$u_x(i,j+1)+u_x(i,j-1) \qquad \textbf{Equation 21.}$$

$$\omega_x = 4[w_x(i+1,j)+w_x(i-1,j)]+\frac{3}{4}[w_y(i+1,j+1)+w_y(i-1,j-1)-w_y(i-1,j+1)-w_y(i+1,j-1)]+$$
$$w_x(i,j+1)+w_x(i,j-1) \qquad \textbf{Equation 22.}$$

$$\theta_y = 4[u_y(i,j+1)+u_y(i,j-1)]+\frac{3}{4}[u_x(i+1,j+1)+u_x(i-1,j-1)-u_x(i-1,j+1)-u_x(i+1,j-1)]+$$
$$u_y(i+1,j)+u_y(i-1,j) \qquad \textbf{Equation 23.}$$

$$\omega_y = 4[w_y(i,j+1)+w_y(i,j-1)]+\frac{3}{4}[w_x(i+1,j+1)+w_x(i-1,j-1)-w_x(i-1,j+1)-w_x(i+1,j-1)]+$$
$$w_y(i+1,j)+w_y(i-1,j) \qquad \textbf{Equation 24.}$$

$$T_x = T(i+1,j)-T(i-1,j). \qquad \textbf{Equation 25.}$$

The dimensionless parameter λ is a ratio of the intra- and extracellular shear moduli

$$\lambda = \frac{\mu}{\nu}, \qquad \textbf{Equation 26.}$$

σ is a length constant characteristic of the bidomain model[3]

$$\sigma = \sqrt{\frac{\nu\mu}{K(\nu+\mu)}},$$

Equation 27.

and Δ is the space step between adjacent grid points. Boundary conditions were implemented by using a layer of fictitious nodes along the boundaries. These equations were solved iteratively for M iterations using the software Octave. In the simulations $N_x = N_y = 103$ and $M \approx 10000$.

RESULTS

Fixed Boundary
The fixed boundary case simulates a sheet of tissue that has all corners and edges constrained such that they cannot undergo displacement, but the tissue is still subjected to a tension T. **Figure 1** shows the intra- and extracellular displacements, **u** and **w**.

(a)   (b)
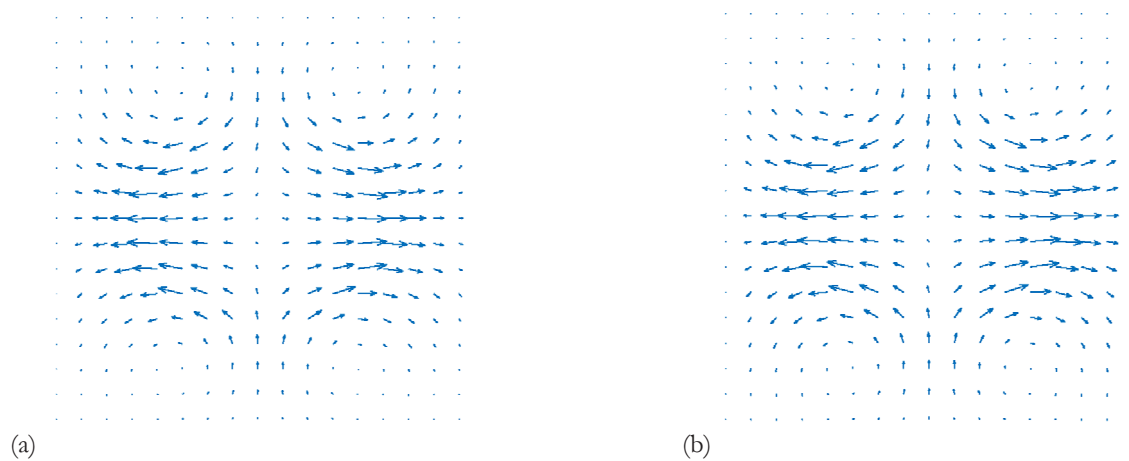
Figure 1. (a) The intracellular displacement **u** and (b) extracellular displacement **w**, for a fixed boundary.

The fiber tension acts in the horizontal direction (the direction of the myocardial fibers) and results in the ischemic border zone being stretched outwards away from the center. The intra- and extracellular displacements are similar.

(a)

(b)
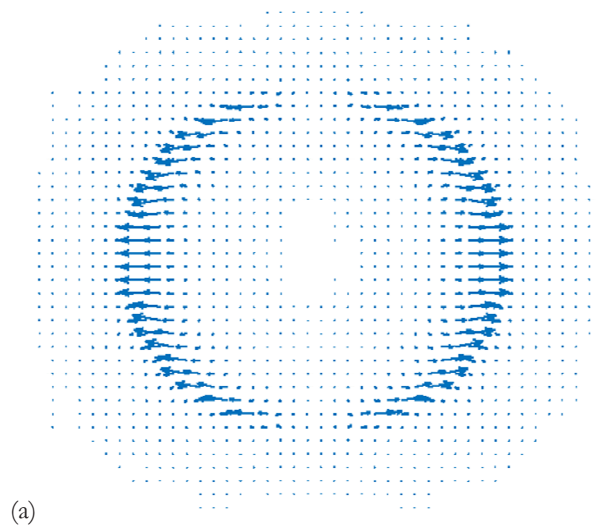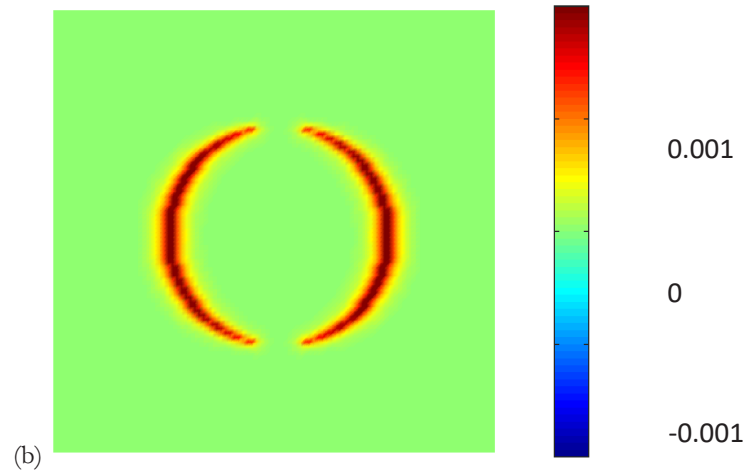
Figure 2. (a) The difference **u** – **w** and (b) the magnitude of the difference |**u** − **w**|, for a fixed boundary.

Figure 2 shows the difference between **u** and **w**. **Figure 2a** is scaled differently than **Figure 1**; the largest arrow in **Figure 1** has a magnitude that is approximately 4 times larger than the magnitude of the largest arrow in **Figure 2**. The difference between **u** and **w** is large only in the border zone, whereas **u** and **w** individually extend into the ischemic and healthy tissue. The difference **u** – **w** is largest in the horizontal direction parallel to the myocardial fibers, and is zero perpendicular to them.
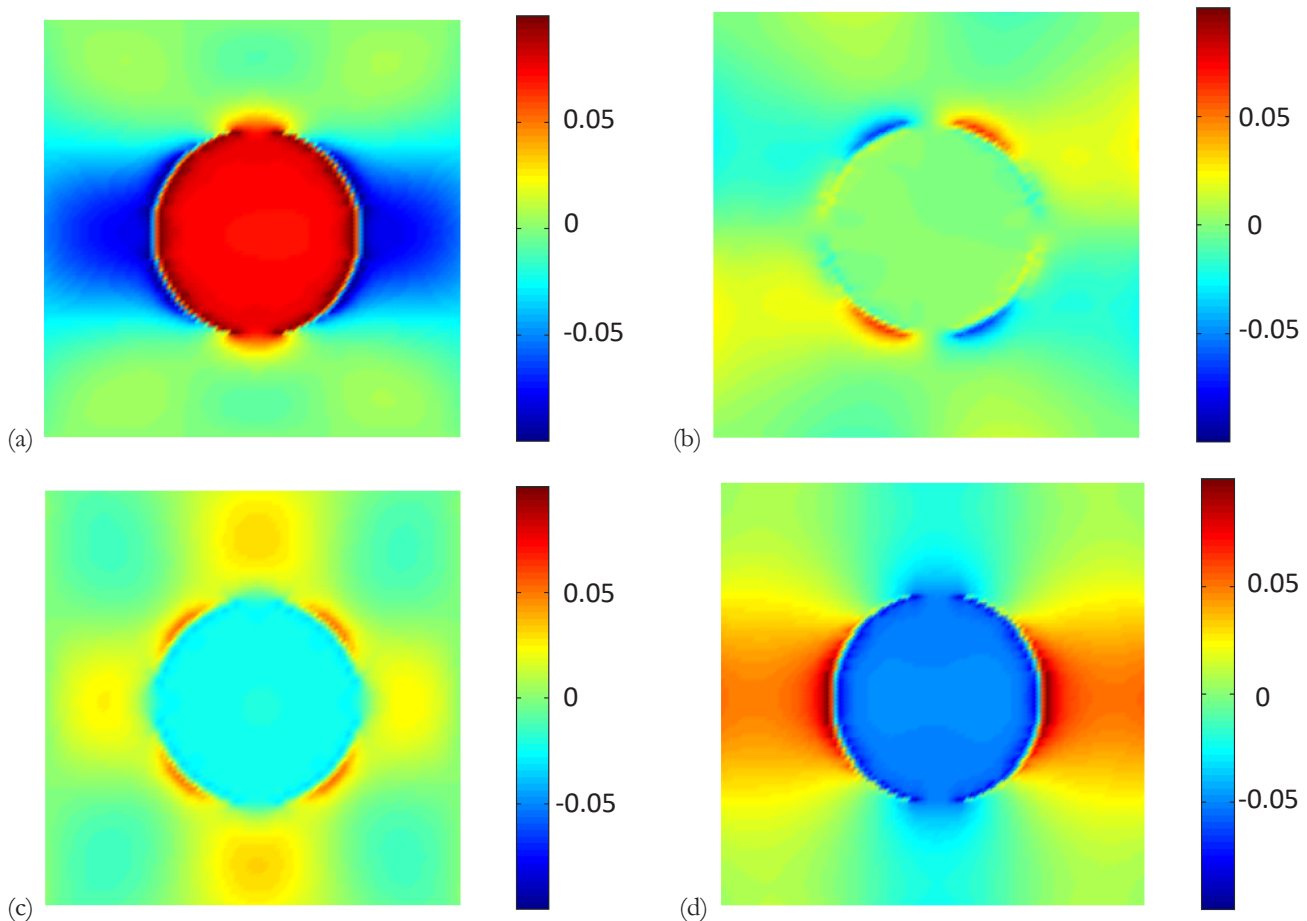
Figure 3. (a) The normal strain (ε_{ixx}), (b) shear strain (ε_{ixy}), (c) the *y*-strain (ε_{iyy}), and (d) the *z*-strain (ε_{izz}) for a fixed boundary.

The strain seems to be largest in the ischemic zone and outside the ischemic region along the fiber direction (**Figure 3**). The displacement difference distribution in **Figure 2** does not match the distribution of the strains in **Figure 3** which indicates that the mechanical bidomain model predicts that mechanotransduction occurs at different locations depending on if strain or membrane force is the causing factor.

Free Boundary
The free boundary case simulates a sheet of tissue that is able to undergo displacement anywhere along the tissue edge. **Figure 4** shows the displacements **u** and **w**. The difference in displacements, **u** – **w**, is virtually zero everywhere except at the vertical edges and in the ischemic border zone (**Figure 5**). The intracellular strains are shown in **Figure 6**.

(a)   (b)
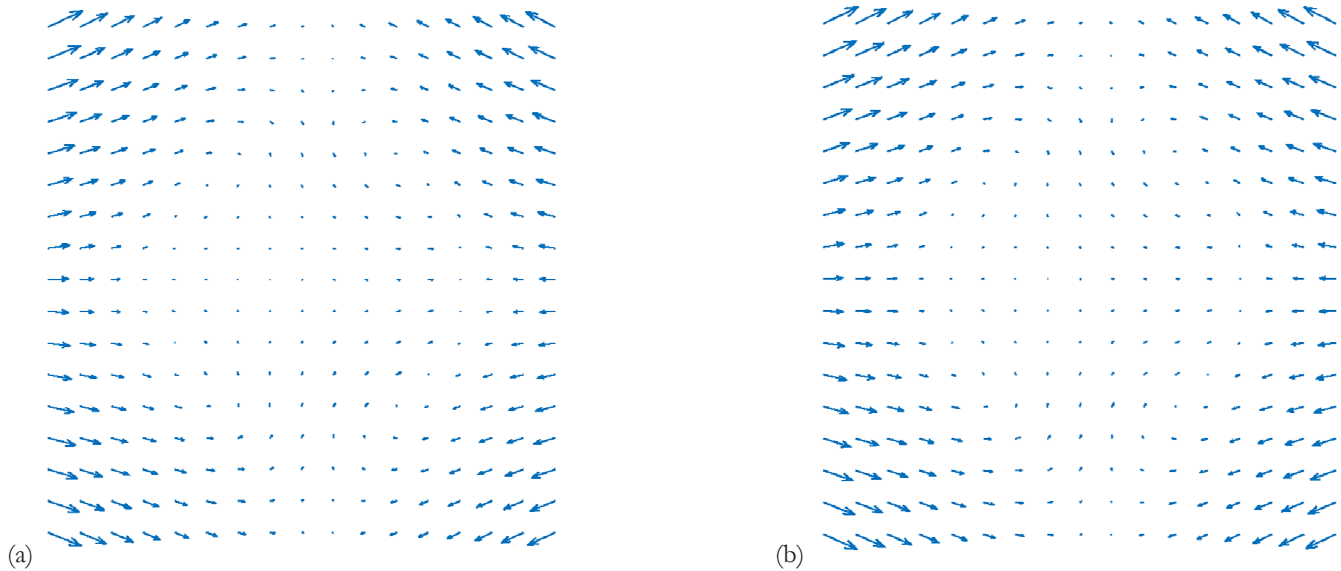
Figure 4. (a) The intracellular displacement **u** and (b) the extracellular displacement **w**, **for a free boundary**.

The displacements **u** and **w** contract inwards to the ischemic region unlike the displacements in **Figure 1**.

(a)   (b)
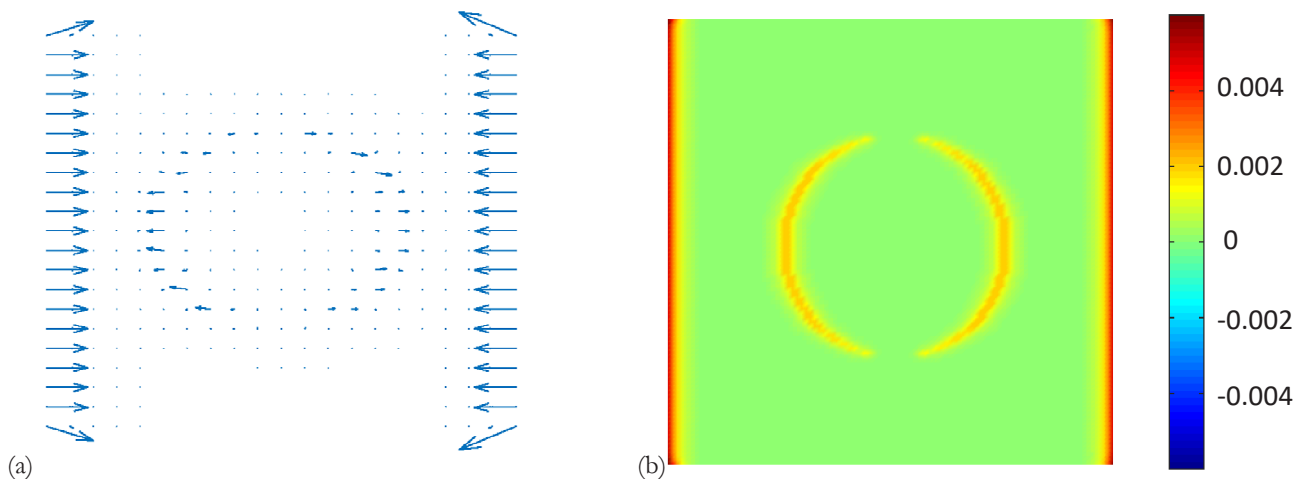
Figure 5. (a) The difference **u** − **w**. (b) The magnitude of the difference |**u** − **w**|, for a free boundary.
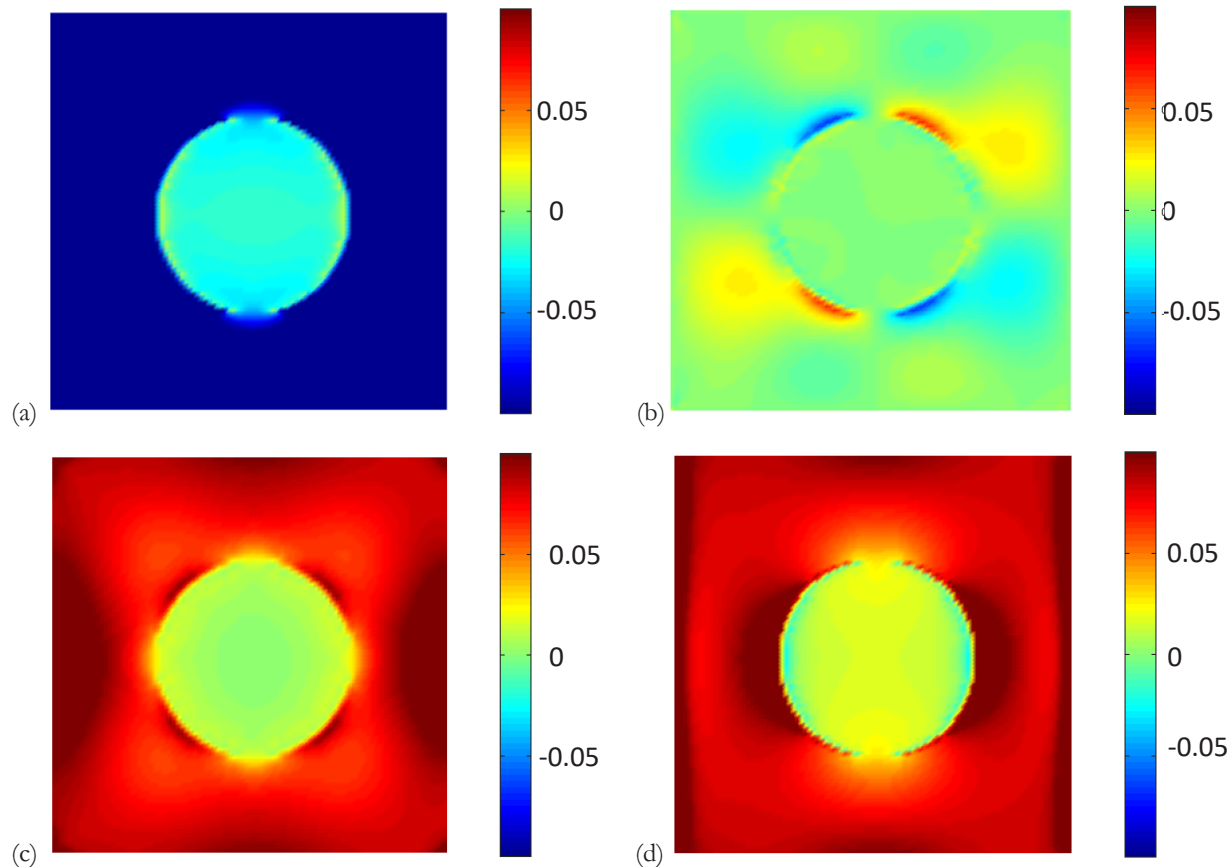
Figure 6. (a) The normal strain (ε_{ixx}), (b) the shear strain (ε_{ixy}), (c) the *y*-strain (ε_{iyy}), and (d) the *z*-strain (ε_{izz}) for a free boundary.

The *z*-strain was largest on the vertical edges with smaller distributions around the ischemic region and zero in the center. The distribution of the *z*-strain is similar to the distribution for the membrane displacement differences along the ischemic region and borders as observed in **Figure 5**, but with a more complicated pattern outside the ischemic region.

DISCUSSION

A previous calculation based on the mechanical bidomain model for an ischemic region in cardiac tissue indicated that stress and strain were distributed widely throughout the normal and ischemic tissue, while the difference in intra- and extracellular displacements was restricted to the ischemic border zone.[6] The localization of tissue growth and remodeling to the border zone was observed clinically.[8] Gandhi and Roth's calculation was based on plane strain: no displacements in the *z*-direction. In this calculation, we compare their results with a similar calculation based on plane stress: no stress in the *z*-direction. In addition, Gandhi and Roth only considered the case of a fixed outer boundary. In our simulations, we consider both a fixed boundary and a free boundary.

Our results for a fixed boundary (**Figures 1-3**) can be directly compared to Gandhi and Roth's results. In both cases, the normal tissue was displaced toward the left and right sides of the tissue sheet outside of the ischemic region along the fiber direction. However, Gandhi and Roth also observed an inward movement of the displacement outside the ischemic region perpendicular to the fibers, caused by incompressibility. This inward movement was not as pronounced in our simulations (**Figure 1**) because with plane stress, incompressibility could be enforced by the tissue getting thinner (displacement in the *z*-direction) as well as the tissue moving inward. **Figure 3d** shows a negative strain in the *z*-direction within the ischemic region, indicating tissue thinning. In both our results (**Figure 2**) and those of Gandhi and Roth, the difference **u − w** was concentrated in the border zone separating the ischemic and normal tissue regions. However, the spatial distribution was slightly different for our calculation of **u − w** compared to that calculated by Gandhi and Roth.[6] Gandhi and Roth found that **u − w** was zero where the border zone intersected the *x*-axis, while **u − w** was largest there in our results. Also, the direction of **u − w** changed; Gandhi and Roth found it to be tangential to the border zone, whereas we found it to be parallel to the *x*-axis. Both calculations found large positive strains ε_{ixx} in the ischemic region, but Gandhi and Roth found large negative strains in the normal tissue both parallel and perpendicular to the

fibers, whilst our results showed only negative strains in the normal tissue parallel to the fibers (**Figure 3**). Therefore, we conclude significant differences exist in the displacement and strain distributions between plane stress and plane strain, although both cases restrict the difference **u** – **w** to the ischemic border zone.

When we allowed the tissue to have a free boundary (**Figures 4-6**), we found additional new behavior compared to the fixed boundary. For instance, the displacements **u** and **w**, and the intracellular strain ε_{ixx}, were nearly zero throughout the ischemic tissue. The surrounding normal tissue contracted (**Figure 4**), but it did not cause the ischemic region to move because there was no fixed boundary to pull on. Unlike the fixed boundary, the tissue expanded in the z-direction indicated by a positive z-strain to obey incompressibility as the tissue contracted. In addition, near the left and right edges of the normal tissue there was a thin boundary layer of **u** – **w**. Such boundary layers have been observed in previous calculations, such as that by Roth.[9] This boundary layer fell off with distance from the boundary exponentially with length constant σ. Therefore, for a free boundary, **u** - **w** was restricted to two regions: the ischemic border zone, and the tissue outer edge. The free boundary strains correlated insignificantly to Gandhi and Roth's fixed boundary strains. They found complex geometries outside the ischemic region where our strain distributions were fairly simple and saturated. Our free **u** – **w** compared to their results similarly as our fixed **u** – **w**, but with the addition of displacement differences on the outer edge. These comparisons further support the difference in strain and membrane force distribution depending on whether plane strain or plane stress is used as well as what boundary conditions are in effect.

The numerical method used in this study differs significantly from that used by Gandhi and Roth.[6] In the case of plane strain, the condition of incompressibity implies that $\frac{\partial u_x}{\partial x} + \frac{du_y}{dy} = 0$, and this condition can be enforced by using stream functions to specify **u** and **w**. For plane stress, the stream functions are no longer useful, and the differential equations of the bidomain model had to be written in terms of displacements instead. Our results are independent of the thickness of the tissue sheet, as long as it is thin enough that the plane stress assumption is applicable. We are primarily interested in the spatial distribution of the strains and membrane forces, rather than their magnitude, so we set T=1. The model is linear, so results for other values of T could be found by linear scaling.

These results have implications for how cardiac tissue recovers from a heart attack. Rodriguez et al. found that mechanical alternations such as remodeling following a heart attack occurs primarily in the ischemic border zone.[8] These results are consistent with our prediction that membrane forces are largest in the border zone.

The plane stress case is useful when analyzing data from experiments on cell monolayers because it simulates a two-dimensional sheet like our model. The free and fixed boundary conditions can be simulated on such monolayers such that plane stress can predict where mechanotransduction will occur.[4,5] Rosowski *et al.* performed such an experiment on a cell monolayer with a free upper surface, but not a fixed one, and found that cells primarily differentiated at the edge.[10] Our model predicted similar results to this behavior. Future experiments simulating a fixed boundary with an ischemic region would offer evidence testing the predictions found using this model.

The model used contained various limitations:
1. The ratio between the intra- and extracellular shear moduli was assumed to be one.
2. The actual value for the length constant is not known.
3. While the z-direction is considered in this analysis, it still does not represent a general three-dimensional solution which would be the ideal model for actual cardiac tissue.
4. Linear stress-strain and strain-displacement relationships are assumed when nonlinearities may exist.
5. Straight fiber geometries are used when realistic cardiac tissue contains curved fiber geometries.[7]
6. The ischemic border zone is abrupt and thin. Actual ischemic borders may be irregular in heart tissue.

In this research, mechanotransduction, under fixed boundaries, was found to reside in the border zone of the ischemic region parallel to fiber orientation. This result is similar to where plane strain models found mechanotransduction to occur under fixed boundaries, but with slight differences in distribution.[6] However, the individual displacement distributions of the intracellular and extracellular spaces between the plane stress and plane strain models differed. These results show that the plane stress and plane strain models localize mechanotransduction to the ischemic border zone, but the distributions of the individual cellular displacements and mechanotransduction differ depending on which model is used. Additionally, the localization of mechanotransduction using plane stress differed depending on if the boundaries where fixed or free showing that boundary conditions affect where cells will grow or remodel on a sheet of tissue. If strain is assumed to be the cause of mechanotransduction then this research found that the strain distributions throughout the sheet of tissue differed depending on if plane strain or plane stress was used. Plane strain, under fixed boundaries, found a more complex distribution of strain throughout the normal tissue than the plane stress model. Furthermore, the distribution of strain under plane stress differed depending on if fixed or free boundary conditions were implemented.

CONCLUSIONS

In conclusion, the mechanical bidomain model found mechanotransduction to occur in the ischemic border zone assuming membrane force as the cause of mechanotransduction while the same model found mechanotransduction to occur throughout the ischemic region and healthy tissue assuming strain as the cause of mechanotransduction. The mechanical bidomain model predicts where mechanotransduction occurs differently depending on whether membrane forces or strains are assumed to induce mechanotransduction. This research may provide valuable insight into understanding how cardiac tissue recovers after a heart attack. Mechanotransduction in cardiac tissue may lead to cellular remodeling.[7] The mechanical bidomain model can be used to describe the remodeling process and predict where the remodeling will occur. These applications of the mechanical bidomain model may aid in describing and understanding in vitro and in vivo experiments dealing with ischemic myocardium. To determine whether cellular strain or membrane force causes mechanotransduction and whether plane stress or plane strain provides a better model for simulating mechanotransduction, more quantitative experiments need to be conducted. The free boundary conditions under plane strain have yet to be analyzed using the mechanical bidomain model. Quantitative evidence for a sheet of tissue with active tension with a central circular ischemic region is lacking. If the conditions used in this research where replicated in a lab and analyzed the results would help determine whether plane stress or plane strain is the better model for predicting mechanotransduction. Additionally, this evidence would also help in determining whether strain or membrane force is the causing factor of mechanotransduction. This experiment could be performed in a lab if a sheet of healthy cardiac tissue was restricted between two coverslips, to simulate plane strain, and was electronically stimulated to replicate active tension. The ischemic region could be induced through drug applications. The plane stress conditions could be replicated if the top coverslip was removed such that the tissue could displace in the z-direction. Fixed boundary conditions could be implemented by pinning the edges of the tissue down so that no edge displacements can occur. Free boundary conditions can be implemented by not pinning the edges of the tissue so that the edges can undergo displacement. Finally, the mechanical bidomain model is only two-dimensional. A three-dimensional model may provide a better representation of mechanotransduction in physical myocardium, but such a model has not yet been derived.

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PRESS SUMMARY
Mechanotransduction is a tissue's biological reaction to a mechanical force. An example of mechanotransduction, relevant to this study, is how cardiac tissue regrows and changes (remodels) following a heart attack. In previous studies, mechanotransduction was thought to be caused by the stretching or shearing of tissue (strain). For this study, mechanotransduction is assumed to be caused by differences in displacements between the intracellular and extracellular spaces producing a force on integrin proteins in the cell membrane. To describe and predict mechanotransduction in tissue sheets, a mathematical model was developed. Earlier studies using a similar model to examine a two-dimensional sheet of tissue assumed the tissue could not move in the direction perpendicular to the sheet (plane strain). This paper examines a different case when the tissue experiences no force perpendicular to the sheet (plane stress). The plane stress case may be a better description of experiments performed on sheets of tissue one cell thick (monolayers). The model predicts that mechanotransduction occurs mainly in the border zone between healthy and unhealthy tissue, and the exact distribution of mechanotransduction differs between the plane stress and plane strain cases.

Potential Consequences of Hosting an Ant-tended Treehopper, *Publilia concava,* for Tall Goldenrod, *Solidago altissima*

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ABSTRACT

In ant-hempteran mutualisms, 'tending' ants indiscriminately defend hemipterans from other arthropods, protecting mutualism-hosting plants from defoliating herbivores in some cases. Censuses of a treehopper, *Publilia concava*, observations of tending ants, and measurements of leaf area were conducted on tall goldenrod, *Solidago altissima*, over the course of a summer at a field site in central Vermont. Hosting ant-tended treehopper aggregations had no effect on leaf area or the ability for goldenrod to flower, suggesting that in the absence of an herbivore outbreak this mutualism is neither necessary nor inherently detrimental for goldenrod. These findings support the hypothesis that the net consequence of the ant-hempteran mutualism for its host plant depends on the costs of hemipteran damage, and the benefits of ant defense from other arthropods.

KEY WORDS

Ants; Mutualism; Ant-plant Interactions; Treehopper; Hemiptera; Ant-hemipteran Mutualism

INTRODUCTION

The consequences of mutualisms for their participants are highly variable and governed by the ecological context in which they occur.[1] In an ant-hempteran mutualism (AHM), 'tending' ants defend hemipterans from natural enemies in exchange for honeydew excretions, which they consume as a food resource.[2] Variability in the consequences of an AHM for its hemipteran participants is well known.[3,4] For instance, the survivorship of a treehopper, *Publilia concava,* inversely relates to the size of its aggregation and the distance of its host plant, tall goldenrod, *Solidago altissima,* from tending ant colonies.[5]

However, AHMs are less studied with regard to variability in the impacts that they may have on their host plants. While indiscriminate antagonism by tending ants may deter other herbivores, such a benefit must outweigh the inherent cost of hosting hemipteran aggregations.[6] Therefore, an AHM may be most beneficial for its host plant when the abundance of other, non-honeydew producing, herbivores is high.[7] Notably, when the density of herbivorous caterpillars was high, AHM-hosting cotton plants produced a greater percent more bolls than non-host plants than when caterpillar density was low.[8]

Messina (1981) reported that two species of chrysomelid beetles, *Trirhabda* spp., occur in high enough densities to completely defoliate tall goldenrod.[9] Under such conditions, tall goldenrod that hosted *P. concava* aggregations most consistently tended by ants were protected from defoliation, grew taller, and were most likely to flower.[9] However, hosting large treehopper aggregations comes at an inherent cost to goldenrod. *P. concava* pierce the midribs of goldenrod leaves and consume phloem sap, potentially lowering the ability for AHM-hosting ramets to photosynthesize.[9] This study examined the potential benefits and detriments for goldenrod hosting an AHM in the absence of an outbreak of *Trirhabda* spp. or similar herbivores. Under such circumstances it can be hypothesized that either there is either no benefit to goldenrod that host an AHM, or that the inherent cost of hosting treehopper large aggregations incurs a net-negative consequence.

METHODS

From June through October 2016, a study was conducted at a field site in Jericho, Vermont (44°27'42" N, 72°59'38" W). The field site is an open field dominated by grasses and perennial herbs and bordered by a mixed deciduous forest along the east and open meadows to the north, south and west. Throughout the field site, *S. altissima* hosts *P. concava* aggregations that are tended by three species of ants: *Formica subsericea*, *Camponotus noveboracensis*, and a species of the genus *Lasius*, most likely *Lasius alienus*. Ants were identified according to Ellison et al. 2012.[10]

In early June, when *P. concava* adults had begun ovipositing on goldenrod, 52 *S. altissima* ramets were marked haphazardly, with an attempt made to include a relatively equal number of ramets that were and were not selected by *P. concava* for oviposition. On 28

June, nymphs had hatched, and from that date until September 12th, censuses were conducted on these ramets approximately weekly. During each census, treehopper adults and nymphs were counted systematically, working from the base of the stem upwards. In mid-September, three leaves nearest to the point three-quarters of the way up the stem from the base[9] were collected from 30 randomly selected ramets: 15 that did host ant-tended treehopper aggregations and 15 that did not. Only 35 ramets from the largest patch of *S. altissima* encompassing an area of approximately 15 m² were considered in the random selection in an effort to control for possible environmental variability among goldenrod patches at the field site. For this purpose, ramets were deemed as having hosted an aggregation if the number of *P. concava* adults and nymphs counted during any one census was greater than 50, an arbitrary value above which most aggregations exceeded. The image editing software ImageJ was used to estimate total leaf area from each leaf (adapted from O'Neal 2002),[11] and the 3 measurements from each ramet were averaged to obtain a single, average value. Towards the end of the flowering season in mid-October, the height of these 30 ramets was measured and their inflorescences were collected. Height was measured from the base of the stem to the highest point of the bouquet while gathering the inflorescence by hand.

Statistical analysis
Of the 30 ramets sampled for leaf area, we considered a ramet as having hosted *P. concava* if we counted at least 15 treehoppers on that ramet during any one census. 15 was used as a cutoff for the statistical analysis, rather than 50, because in fact one ramet with a maximum count of 15 treehoppers had hosted a small *P. concava* aggregation. On ramets where the maximum count of treehoppers during any one census was lower than 15, *P. concava* presence was circumstantial and not due to oviposition and the establishment of an aggregation. Thus, the statistical analysis included 16 *S. altissima* ramets that hosted ant-tended treehopper aggregations and 14 that did not. Average leaf area was treated as a response variable in an analysis of covariance (ANCOVA), with the main effect as treehopper aggregation presence. Height was treated as a covariate, because leaf area and height were positively correlated (Linear Regression, Adjusted $R^2 = 0.1362$, $p < 0.05$). All analyses were conducted in R version 3.4.4.[13] using code from Mangiafico 2015.[14] Figures 1 and 2 were created in R using the package 'ggplot2'.[15]

RESULTS
P. concava aggregations peaked in number during mid-July (**Figure 1**). The largest number of adults and nymphs recorded on any single *S. altissima* ramet during any one census was 502 individuals. *Formica subsericea* and *Camponotus noveboracensis* tended treehoppers on the same ramet concurrently, and *Lasius* sp. constructed fragile 'shelters', apparently made of soil, around the treehoppers they were tending. This behavior has been described before by *Lasius alienus* to protect lycaenid caterpillars,[16] but to the authors' knowledge this is the first time that it has been reported to occur on tall goldenrod to protect *P. concava*. The presence of *P. concava* aggregations did not have a significant effect on leaf area (ANCOVA, $F_{1,27} = 0.3231$, $p = 0.57$; **Figure 2**), and all 30 ramets, AHM-hosting and non-hosting alike, produced inflorescences.
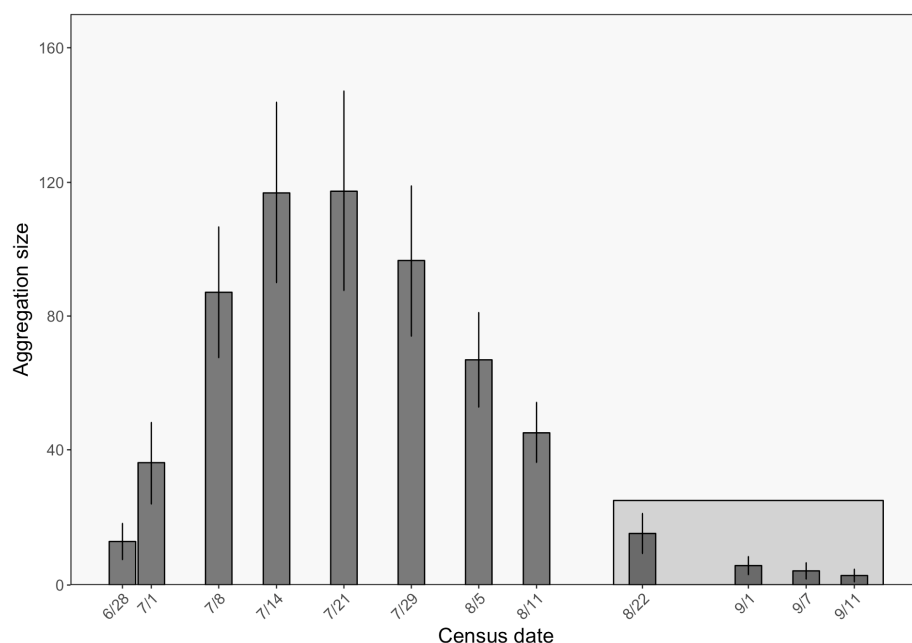
Figure 1. Mean aggregation sizes of *P. concava* treehoppers among 16 *S. altissima* ramets that hosted ant-tended aggregations during the summer of 2016. Censuses conducted when inflorescences were in bloom are in the highlighted box. Vertical lines represent standard error.
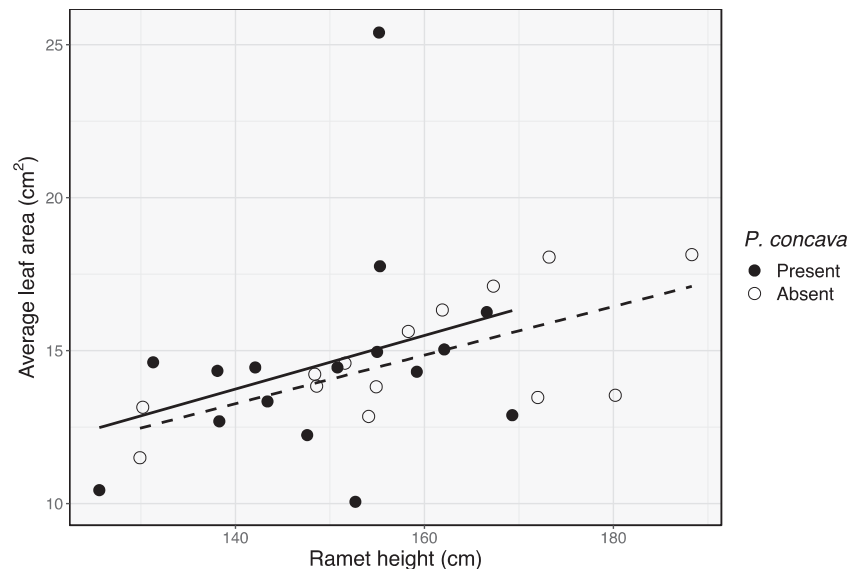
Figure 2. Relationship between the leaf area and height of 30 *S. altissima* ramets with the plotted linear regression fits for ramets that that did (solid line) and did not (dashed line) host ant-tended *P. concava* treehopper aggregations.

DISCUSSION

Messina (1981) reported that under severe herbivory from leaf-chewing beetles, *Trirhaba* spp., goldenrod hosting *P. concava* aggregations most consistently tended by ants had the highest likelihood of flowering, and subsequently setting seed.[9] In this study, goldenrod ramets were under little threat of severe defoliation and, indeed, there was no significant difference in leaf area between ramets that hosted AHM and those that did not. Under these circumstances, there was no evidence that the presence or absence of an AHM impacted the ability for goldenrod to flower. However, it is important to consider other key factors that may impact how the presence of *P. concava* influences the performance of goldenrod and present opportunities for further study.

On goldenrod ramets hosting treehopper aggregations, it was clear that feeding by *P. concava* damaged goldenrod leaves, causing them to droop at the site where they were initially pierced and brown more quickly than undamaged leaves (**Figure 3**). It would be useful to directly measure this damage to AHM-hosting ramets and its implications. The effects on photosynthetic rate by other goldenrod herbivores have been studied in a greenhouse setting, finding that only a spittlebug, *Philaenus spumarius*, significantly reduced the photosynthetic and growth rates of goldenrod.[17] Among the insects examined in that study, which also included aphids and *Trirhabda* beetles, *P. concava* is most closely related to the spittlebug. A similar greenhouse study may find that when treehopper aggregations are extraordinarily large, they have a similar effect on AHM-hosting goldenrod that translates to a net-negative consequence for flowering success.

Figure 3. Aggregations of the treehopper, *P. concava*, damage the leaves of goldenrod hosts by piercing their midribs and consuming phloem. Damaged leaves droop at the site of feeding and brown more quickly than undamaged leaves.

At the field site, the presence of *P. concava* may not be the most important insect affecting the overall performance of goldenrod. Upon inspection it became apparent that case-bearing moth larvae (Lepidoptera: Coleophoridae) had consumed seeds of goldenrod inflorescences. It would be intriguing to discern whether these seed predators or defoliation by *Trirhabda* spp. beetles has a greater effect on reproductive success, and if ultimately, hosting an AHM is of significant benefit to goldenrod in terms of relative fitness. It is also important to recognize that since *S. altissima* is clonal, it is possible that the ramets in this study represented a single clone, and that the ability for ramets to share resources may protect a clone from *P. concava* from extensive damage as a whole.[18] A study incorporating genetic analyses and comparing the mutualism's impacts among separate patches of *S. altissima* over a larger spatial and temporal scale would be tedious, but reveal a more accurate and entire representation of the net effects of hosting an AHM for goldenrod.

CONCLUSIONS

Underscoring the importance of considering the ecological context in which mutualisms occur, this study considered the infrequently tested hypothesis[6,8] that that the benefits of hosting an AHM may depend on factors such as the abundance of other herbivores. [8] These findings suggest that examining the effects of an ant-tended treehopper on its host's photosynthesis, and further investigating the net effect of hosting an AHM in the context of herbivores other than defoliating beetles, such as lepidopteran seed predators, may prove useful in elucidating the conditions under which AHMs are beneficial as well as costly.

ACKNOWLEDGEMENTS

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Luke McCartin graduated from the University of Vermont in May 2017 with a Bachelor of Science in Biological Sciences. Following graduation, he joined the Biology Department at the Woods Hole Oceanographic Institution as a research assistant, and he plans to pursue a graduate degree studying biology and conservation.

PRESS SUMMARY

Ants and treehoppers engage in a common mutualism when ants defend treehoppers from other insects in order to consume their sugar-rich honeydew excretions. This species interaction may be vital for goldenrod to flower in some instances, because ants indiscriminately attack many insects, including herbivorous beetles. We observed the mutualism on goldenrod at a field site in Vermont over the course of a summer, and consider the implications of hosting treehoppers for goldenrod in the absence of defoliating beetles or similar herbivores.

Using Smart Glasses for Facial Recognition

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ABSTRACT

Facial recognition is one of the most promising applications of smart glasses and can help many organizations become more efficient. For example, police traditionally identify criminals by manually going through pictures in a database which makes face matching a slow process. However, with the combination of facial recognition software, smart glasses, and databases, the police can quickly scan through multiple databases of faces to find a match. The police would also be able to spot criminals in crowds, identify unknown victims at crime scenes, retrieve background information on individuals, and verify if someone is a missing person. The Transportation Security Administration (TSA) can also use this combination to identify potential terror suspects or verify the identity of travelers. Lastly, academia can benefit from these tools by being able to identify individuals at events (*e.g.* conferences) and display relevant information about them. The goal of this project is to write an Android program that takes a photo via Google Glass, compares it with a predefined sample database held within the smartphone, and outputs information based on its analysis. The results are displayed with an accuracy acceptance level to the user both on their Android smartphone and on their Google Glass.

KEYWORDS

Face Detection; Facial Recognition; Smart Glasses; Android Smartphone; Mobile Application; Google Glass; Java; SQLite

INTRODUCTION

Facial recognition is the process of capturing an image, analyzing it, and identifying someone based on that still, video, or live image of their face. It is not new to the world of cybersecurity and law enforcement. Facial recognition is becoming one of the most popular surveillance technologies and it is being increasingly used by law enforcement. It has evolved to the point where it can be used in both photos and videos to find a match in a database of faces. A step beyond simple facial recognition would be facial recognition combined with smart glasses technology. Law enforcement can benefit from this combination because it can help them determine if someone is a person of interest even if they are hidden in a crowd. However, there is still a debate about privacy when using this technology. The authors understand the importance of privacy so this research focuses on recognizing only the student authors' images and the purpose of this research is for educational use only.

Real World Examples

Facial Recognition software on its own is available through multiple companies. Amazon developed their own facial recognition Application Programming Interface (API) known as Rekognition that can recognize faces and objects, however, it comes at a cost.[1] Microsoft also offers their own facial recognition API called Face, however, depending on the number of facial recognition transactions you want to perform, you also have to pay a fee.[2] A quick Google search will show more companies marketing their own facial recognition software, but the cost factor associated with using their software may deter people from exploring the world of facial recognition. One algorithm that performs facial recognition and does not come at a cost was created by OpenCV.[3] Their facial recognition code is free for academia.[4] OpenCV is a library that is focused on real-time computer vision programming functions and is mainly written in C++.[5] A counterpart to OpenCV is JavaCV.[6] JavaCV uses JavaCPP Presets which acts as a bridge between OpenCV's C++ code and Java code.[7]

Facial recognition is already being used by law enforcement. According to The New York Times, when a suspect for the 2018 Capital Gazette newsroom shooting refused to identify himself, the police used facial recognition to identify him.[8] The New York Times also noted that facial recognition tools are becoming standard for law enforcement in the United States.[8]

While facial recognition alone is a great tool, being able to use it in combination with smart glasses makes it even more useful to law enforcement. There is already a real-world example of combining facial recognition and smart glasses. LLVision is a Chinese company that has created such a combination[9] and has allowed Chinese law enforcement to identify car license plates and faces based on a database while the police are in the field.[10] Being able to use smart glasses allows the Chinese law enforcement to have a hands-free tool that can quickly and accurately identify someone. As long as the technology is used to protect citizens and not abused, facial recognition can be extremely beneficial.

Related Works

Researchers at University of Oulu, located in Finland, created a program that can perform real-time face detection and recognition while running independently on smart glasses.[11] However, while their smart glasses can perform all the face detection and recognition, their program can only display the face position and identifier and did not include additional information about the person.[11]

The Technical University of Munich, located in Germany, created a cognitive assistant application that also uses real-time facial recognition and runs on smart glasses.[12] Their application uses fog computing which, they stated, is the process of inserting a fog layer between the client and the cloud server in order to use less power and run faster than other real-time applications.[12]

This Study

The Android application created for this project is designed to receive a photo via Google Glass, compare it with a sample database held within the smartphone, and display the results to the user on their Google Glass and Android smartphone. The application also accounts for partial matches by giving the user an acceptance level. Depending on the acceptance level, the user will be informed if there is a match, potential match, no match, or no face detected. The results will be sent to the user's Android smartphone and smart glasses via a notification. **Figure 1** is a simplified visualization of the interaction between the Google Glass and the Android smartphone. The application is called Face2Rec because it stands for Face to Recognition and this paper will give an overview of how it was created and provide insight into its code. This study will also look at the reliability of our chosen facial recognition algorithm.
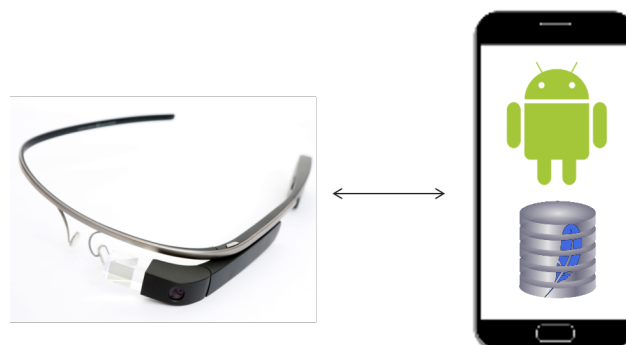
Figure 1. Interaction between smartphone and Google Glass.

Based on our research of all the facial recognition algorithms, APIs, and tools available, JavaCV was selected due to cost factors and its compatibility with Android Studio. The application was written using Java code, so using OpenCV indirectly through JavaCV was advantageous. This is due to OpenCV being written in C++ which means using it in Android Studio, which supports Java programming, adds extra complexities. Also, as undergraduate researchers delving into facial

recognition for the first time, OpenCV and specifically JavaCV libraries are very beneficial because they allowed the authors to develop and learn basic concepts of face detection and recognition without worrying too much about creating a face detection and recognition algorithm from scratch. **Figure 2** shows an overview of how the application works.
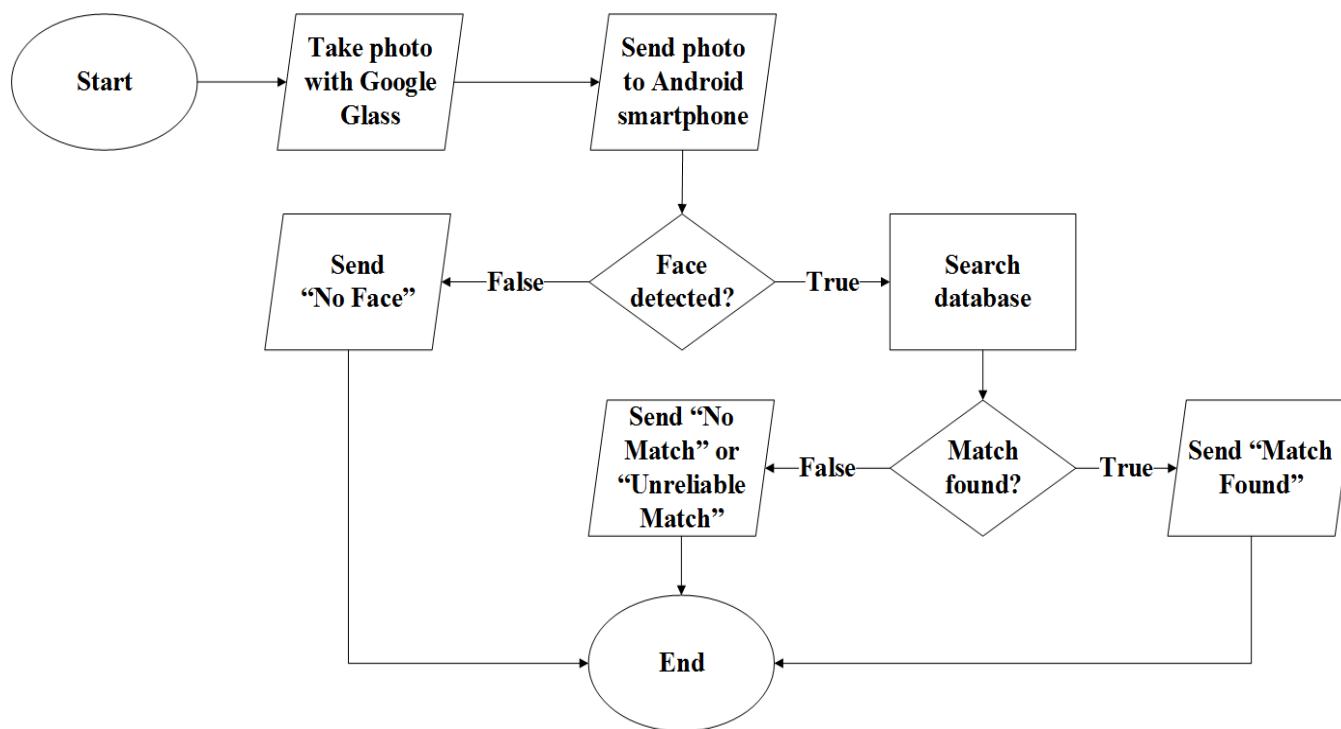
Figure 2. Overview of how Face2Rec works.

TOOLKIT

Our toolkit comprised of five different components as shown in **Figure 3**. The authors relied on OpenCV and JavaCV for the face detection and facial recognition algorithms. Android Studio is used as the integrated development environment (IDE) to do all the programming in Java. SQLite, the database management system, is used to hold all the match information. With regards to hardware, a Samsung Galaxy S9 is used as the smartphone and Google Glass is selected as the smart glass.

Figure 3. The tools that were used.

FACE DETECTION AND RECOGNITION IMPLEMENTATION

Face Detection

The image file type used for this project is called a bitmap. However, the raw image that is saved in the smartphone may not be in a bitmap format so it must be converted. Once a bitmap image is available on the smartphone, it must be converted to Mat. A Mat is a class that can contain a color or grayscale image that is broken down into an array of numbers.[13] OpenCV must first take the color image and turn it into a color Mat. After OpenCV has the color Mat, it can take it and finally convert it to the grayscale Mat.[14] A grayscale Mat is needed for the face detection to properly work. Now that Face2Rec has a grayscale Mat of an image, it can use OpenCV's CascadeClassifier class to detect objects within the selected

data.[15] The function loadClassifierCascade loads the frontalface.xml classifier file. In order for Face2Rec to detect faces in an image, the Haar Feature-based Cascade Classifiers is used.[16] Once Face2Rec finds all the faces in an image, a green rectangle is placed around the detected faces. **Figure 4** shows a flowchart of the face detection algorithm implementation and how the results display on the screen.
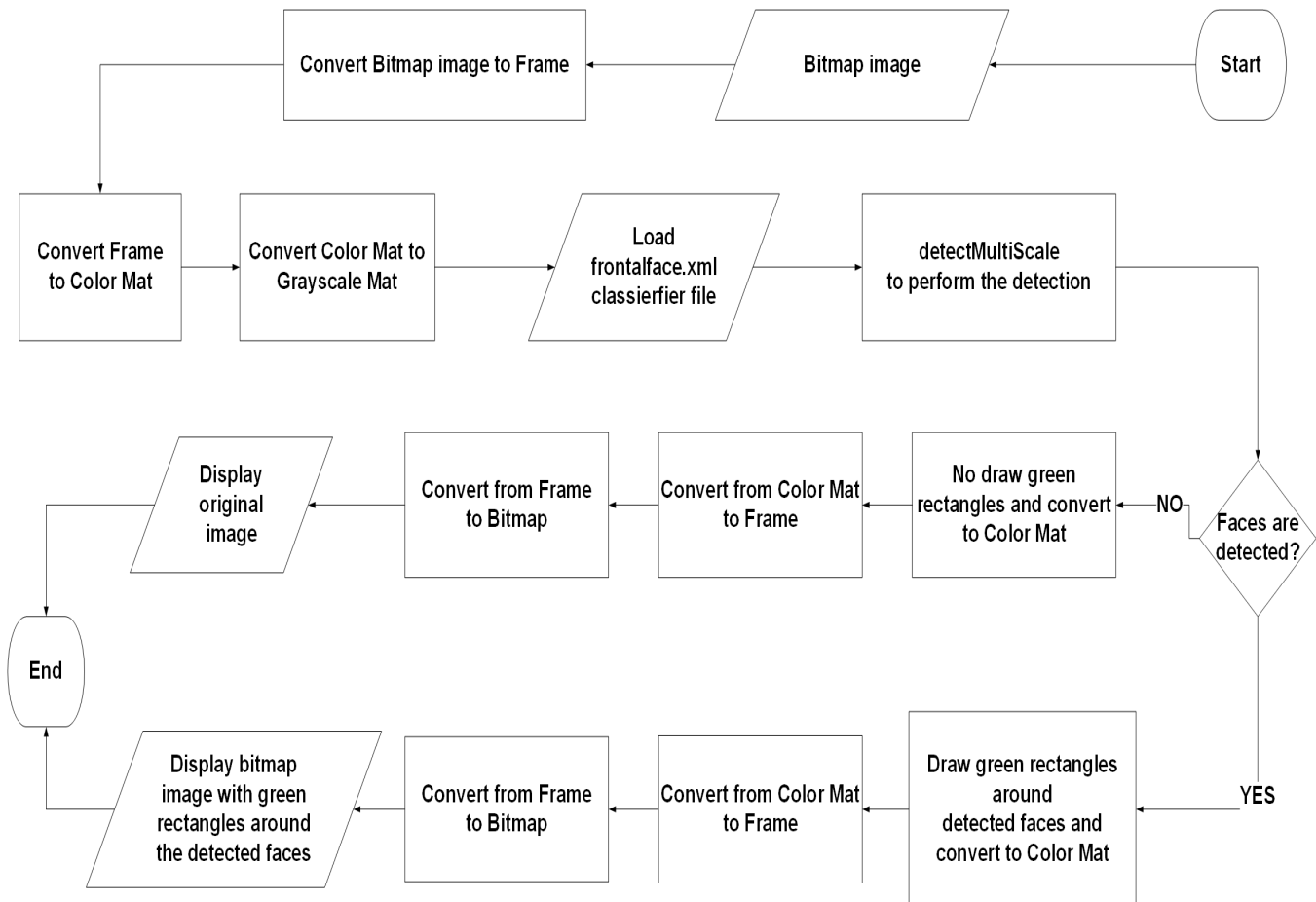
Figure 4. The face detection and display flowchart.

Model Creation

A functionality that is not released in the final version of Face2Rec is model creation. Model creation is the process of saving photos in a specific folder on a Samsung smartphone that adheres to OpenCV/JavaCV's model rules. An example of a model rule is that the photo must be in grayscale. Model creation is a feature of OpenCV/JavaCV that users who are not equipped with the background in OpenCV/JavaCV may mishandle. This is due to the need to use Android Studio to ensure the Face2Rec code can link to the model and ensure the database matches with the correct model. The process of model creation will still be explained here for future reference. After implementing face detection and capturing all the detected faces in a photo, a model will be created of each individual. For this study, the images used to create models belonged to the student authors. Through our testing, it was found that using more faces for training made the facial recognition more accurate. As a result, the model has about forty images for each student author. Initially, our images were captured using the Google Glass and then the images were automatically sent to the gallery. After that, our selected images were detected and stored in a training folder located in the external storage of the smartphone with the correct grayscale format and custom unique names. Finally, by using the create and train methods of EigenFaceRecognizer class,[17] a model of our faces is created using all the images in the training folder. The model is saved in the same folder as the training images. The EigenFaceRecognizer class has methods available in both OpenCV libraries and in Bytedeco's JavaCV.[18] To elaborate further on EigenFaceRecognizer, it has a create method that creates an object of the FaceRecognizer which con-

tains the facial recognition algorithms.[19] The EigenFaceRecognizer's train method takes in two parameters, the trained images and the labels that correspond to the images. The labels are the keys for the facial recognition implementation. In other words, each person has a label attached to them and people are recognized by their different labels. **Figure 5** shows a flowchart of our model creation.
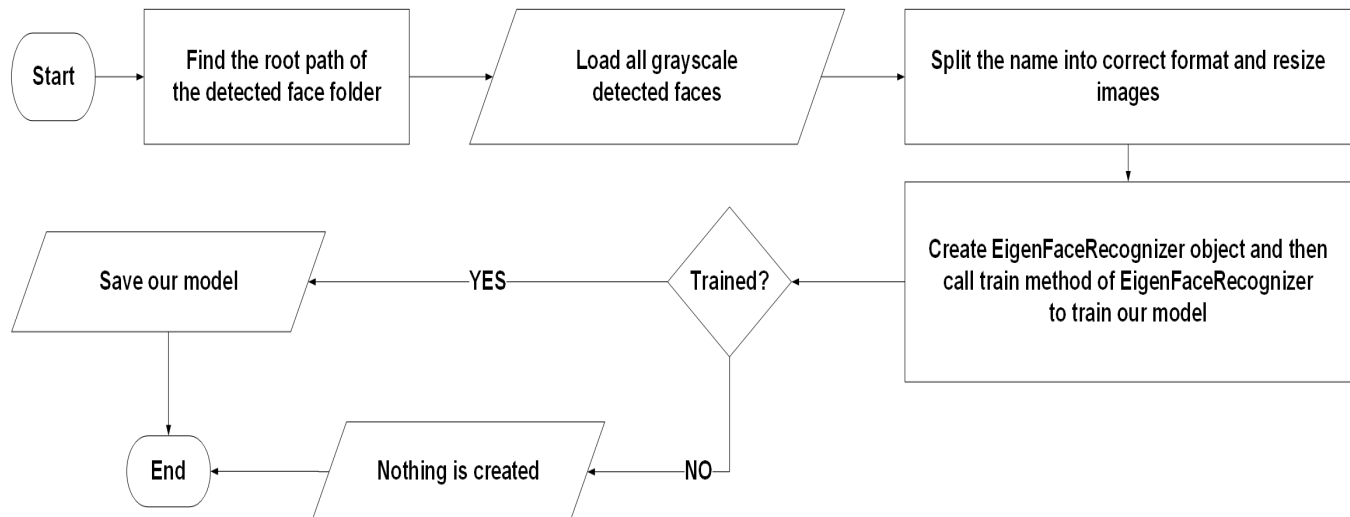
Figure 5. The model creation flowchart.

Database Setup

After creating a model, the SQLite external database is created using Android SQLiteAssetHelper.[20] Each person has an ID number as their primary key, a second field which contains their name, and a third field which contains their general background information. Creating and modifying the database was not hard because of the simplicity of SQLiteAssetHelper. As a result, the authors could concentrate on the more important parts of the application which was the facial recognition implementation and the communication between the Google Glass and smartphone.

Outcome

After creating the model and database, the EigenFaceRecognizer class was used to implement facial recognition. It utilized a prediction number and acceptance level number to match faces in a photo to faces in the models. Every image is assigned a specific label before the training, so when JavaCV's predict function is called, the label from a matching face was received from the model. By getting the label of the matching face, the prediction number was available and the correct matching face was known.

The acceptance level is a number that tells the user how reliable the results are. The closer the number is to zero, the more reliable the results. For best results, the acceptance level is set to the lowest number that would perform the best accuracy. The acceptance level was determined by trial and error. Face2Rec was tested multiple times to decide what would be the lowest possible acceptance level that is still accurate. If an acceptance level is higher than 4000, then the subject will be listed as unknown. If the acceptance level is greater than or equal to 3500 and less than or equal to 4000, then that is a middle acceptance level and the subject is a potential match. Lastly, if the acceptance level is less than 3500, then that is a low acceptance level and the match is reliable. If Face2Rec indicates a positive match, then the closer the acceptance level is to 3500 the more the user must scrutinize the results to determine its accuracy. **Figure 6** shows the facial recognition and display flowchart.
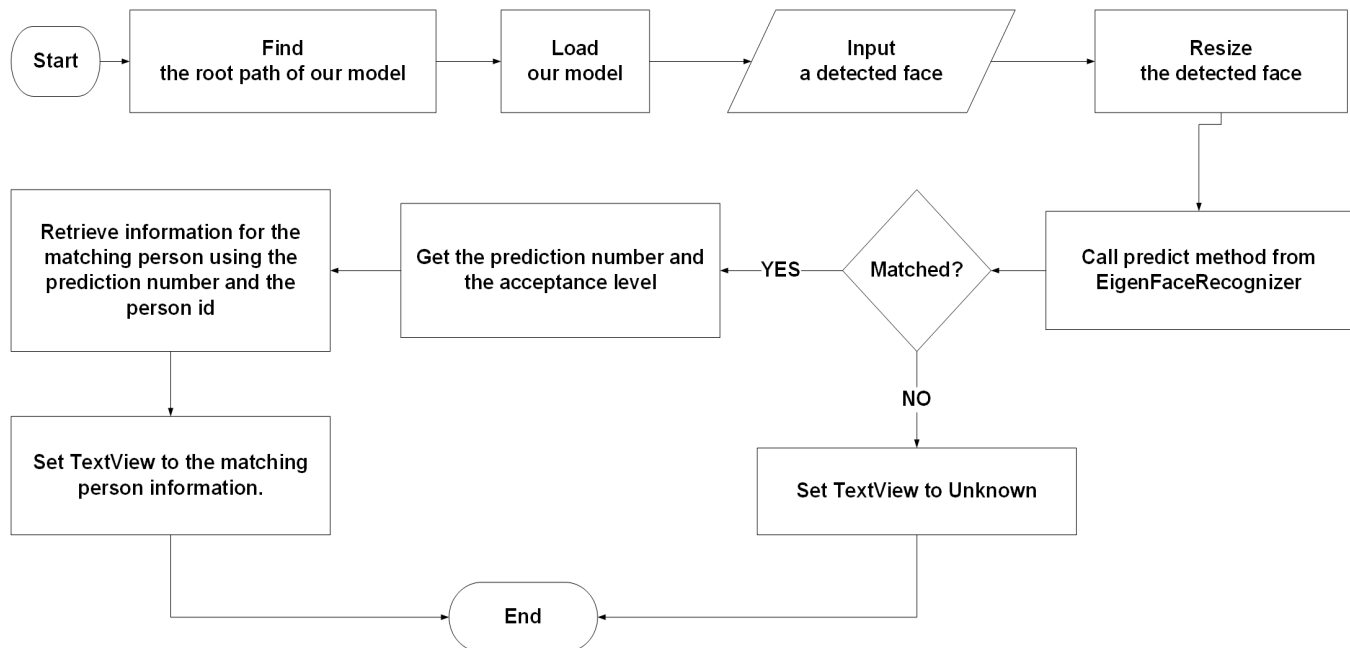
Figure 6. The facial recognition and display flowchart.

COMMUNICATION BETWEEN SMART GLASSES AND SMARTPHONE

Notifications Overview

Android provides online developer guides in order to educate users on how to program using Android Studio.[21] Within Face2Rec's code, more than thirty classes, packages, *etc.* are imported such as NotificationCompat,[22] to send notifications, and bitmap,[23] an image file type, in order to create this project. The NotificationCompat can only process bitmaps, so in order to send notifications, all imported images from the smartphone's gallery will be converted to bitmaps. The function notifications()[24] is programmed to use NotificationCompat so it was possible to tap into Google Glass' built-in feature of displaying notifications from a connected smartphone.[25]

Detecting Latest Image in Gallery

When Face2Rec first opens, it will check for images in the gallery by running the function lastPhotoInGallery() and display the latest photo in the gallery[26] or a blank main menu if there is no photo. The function moves a cursor to first position using moveToFirst() in order to detect for images in the gallery. Face2Rec uses the class GalleryObserver based on The Engineer's Cafe's DirectoryFileObserver[27] which extends the Android class FileObserver[28] in order to detect if there are changes in the smartphone's gallery. However, our GalleryObserver checks for modifications instead of if something was created. The Google Glass will automatically send any photos it takes to the smartphone's gallery and the Gallery-Observer is constantly checking for those photos. A Handler was required to create a delay[29] to check for images because, based on code testing, the new images would not be detected by the GalleryObserver without a slight delay of two seconds. Once an image is detected in the gallery, BitmapFactory's decodefile[30] function converts the image into a bitmap for processing. That bitmap photo would be saved into a variable called bitmapAutoGallery.

Sending Latest Image as a Notification

In the function lastPhotoInGallery()[26], if there is an image and bitmapAutoGallery is updated, then that image will be sent to the function detectDisplayAndRecognize() to go through face detection and recognition. There, if bitmapAuto-Gallery has no faces, then it will go to the function notifications(). If there are faces detected, then the image will go to the function recognizeMultiple(), then displayMatchInfo(), and finally the image would be sent to notifications().

Opening the Gallery

Another option that is available for the user is to use a gallery viewer which displays all photos in gallery mode using the openGallery() function.[31] The user is shown a button on the main screen called "Select A New Face To Recognize". The images displayed are taken from the Google Glass since the Glass photos are delivered there directly. The MyGlass application provides this photo sync option and is allowing direct communication from the Google Glass to the Android smartphone.[32] In the function onActivityResult(), selected photos are converted to bitmaps[33] and saved under a variable called bitmapSelectGallery. If the user selects to open the gallery, but does not choose a photo, then an alert will appear stating no gallery image selected. If an image is selected from the gallery and bitmapSelectGallery is not null or empty, then the image will go to the function detectDisplayAndRecognize(). There, if no faces are detected, the image will go to notifications(). If there are faces detected, then the image will go to recognizeMultiple() and then displayMatchInfo(). Lastly, the image would be sent to notifications(). The two options users have to send their photos through Face2Rec's code is shown in **Figure 7**.
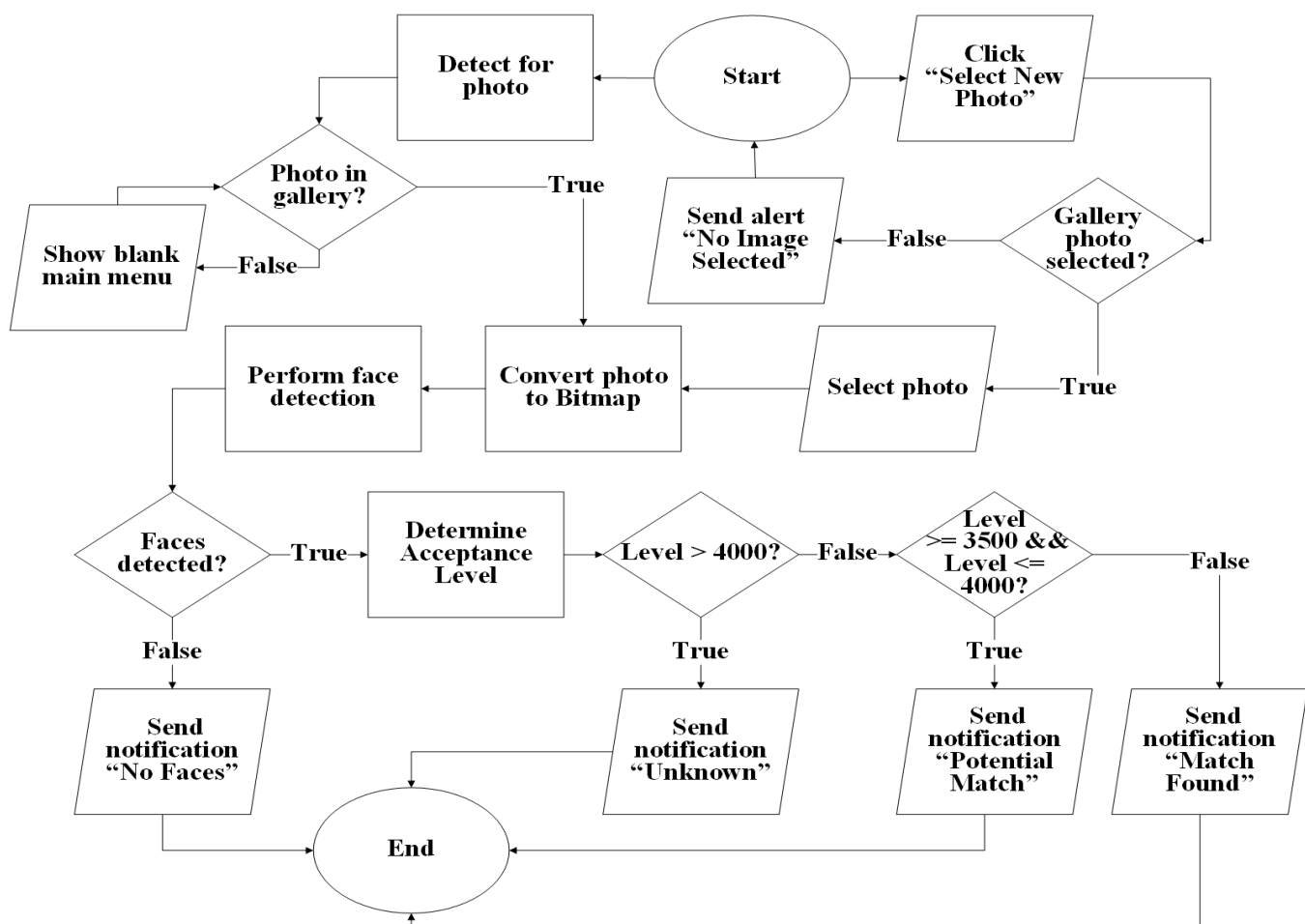
Figure 7. The notifications flowchart.

Sending Notification Images

Now that it is established how images are sent to notifications(), Face2Rec must decide which image to display when sending notifications. This is done by a simple if/else statement that checks to see if bitmapSelectGallery and bitmapAutoGallery are not null. At the end of running notifications(), bitmapSelectGallery is set to null in order to free the variable for a new selected image. So if bitmapAutoGallery is not null, then the image gets saved into a variable called finalBitmapPic, but if bitmapSelectGallery is not null, then that image will be saved in finalBitmapPic. The bitmap variable finalBitmapPic is required because NotificationCompat can only accept one variable in its method setLargeIcon. This method

displays images to the smartphone and Google Glass once a notification is sent. The application knows what text to display based on what is saved in the variables matchText and moreInfo that was defined in the function detectDisplayAndRecognize() if there are no faces detected or displayMatchInfo() if there are faces detected. The text that is displayed is pulled from a previously created database. If the text that is saved in the database is too long for the smartphone screen, then the text will display with the option to scroll through the text on the main menu. This was done by setting scrollbars to equal vertical in the TextView under layout. The notification that appears on the smartphone can also be tapped in order to expand the notification. The Google Glass automatically enables a scrolling feature to view more information of any notification that is sent to it. To enable the scrolling feature, the user only needs to tap the Glass when the notification appears.

SETTING UP THE DEVICES

Downloads

To begin using Face2Rec, the user must download the Google Glass MyGlass[34] application from the Google Play store and download Face2Rec onto their smartphone. Our Face2Rec application is not available on the Google Play store, so the user must add it via Android Studio. They can do this by cloning Face2Rec from Github[35] into Android Studio and then running it on their Android smartphone. Preferably, their smartphone will be similar to our Samsung Galaxy S9 due to the processing power the two applications will require. The MyGlass application enables the user's smartphone to connect with their Google Glass and the Face2Rec application will be where the face detection and recognition will occur. After downloading both applications, the user must set them up.

Linking Devices

On the MyGlass application, the user must link the Google Glass to their phone by following the Google Glass instructions that are displayed on the Google Glass and MyGlass application. In order for Face2Rec to work, the user must enable Notification Sync and Photo Sync in MyGlass. **Figure 8** shows the icon for MyGlass and the settings option[34] on MyGlass that enables Notification Sync. The Photo Sync option may display on the main page instead of in settings. Notification Sync is required so Face2Rec's results can be displayed onto the Google Glass. Photo Sync is required so the Google Glass can save images to the user's smartphone gallery. The user must also allow Face2Rec to have read/write privileges to their device which can be done once the application first launches on their smartphone or in their phone's settings when they download the application.
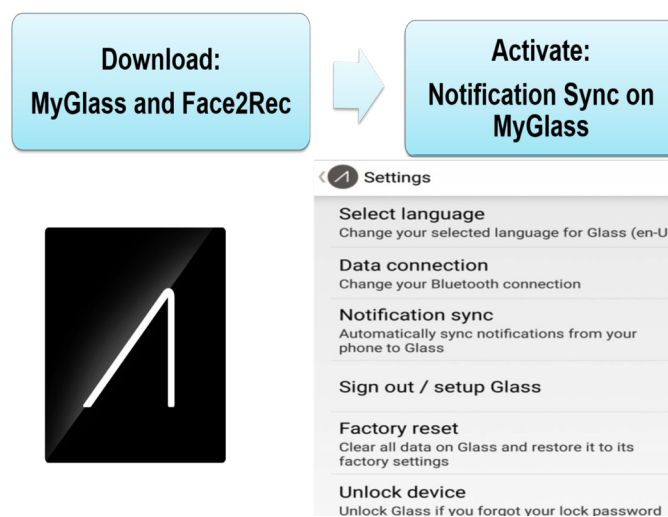
Figure 8. Setting up Notification Sync.[34]

USER EXPERIENCE

Main Menu

Once the user has set up both applications, their smartphone, and the Google Glass, they can then begin to use Face2Rec to perform facial recognition. Upon opening Face2Rec, the user will see the most recent photo on their device displayed on the main menu page or a blank menu if there are no photos. **Figure 9** shows what the user may see. Face2Rec will automatically search for a match based on what is on the main menu and display one of four outcomes to the user: no match, potential match, match, or no face detected. When the user takes a new photo with the Google Glass, Face2Rec will automatically detect a new photo has been added to the smartphone via the Google Glass MyGlass application and update the Face2Rec application to begin its facial recognition process. The user also has the option to manually open their phone's gallery via Face2Rec to select a photo for facial recognition processing.
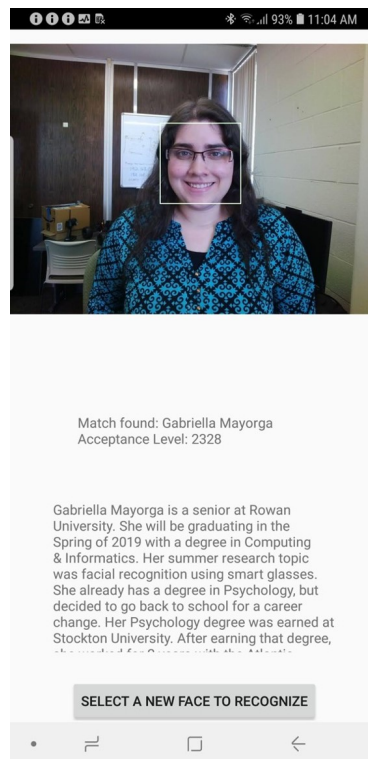
Figure 9. Face2Rec main menu.

Outcome

If Face2Rec performs its facial recognition processing and finds a match, information about the match that is stored in the database will be displayed on both the user's smartphone and Google Glass via a notification and on the main menu. If there is no match, partial match, or no face detected then the user would be sent a notification with that information. The user will also be given an acceptance level in order to determine the reliability of the information that is being displayed. The acceptance level is set in the recognizeMultiple() function and the displayMatchInfo() function determines how to categorize the level. The higher the acceptance level, the less reliable the match. More information about acceptance level can be found in the *Face Detection and Recognition Implementation* section. While an acceptance level may be worryingly close to 3500, the user must determine if glasses, hair being in the subject's face, or anything else affected the level before deciding the accuracy of the results.

Scrolling and Dismissing Notifications

Google Glass and Face2Rec are equipped to handle long text once a photo is done being processed for a match. If the text sent to the user about the processed photo is too long to fully display on either the smartphone or Google Glass, then the

option to scroll through the text is available. Face2Rec allows the user to scroll directly on the application using touch, notifications on the smartphone can be opened to accommodate more text, and the Google Glass also gives the user the option to open the notification as scrollable text right on the Google Glass display. Once the user is done viewing the notification, they can dismiss it from their screen. If they wish to not dismiss the notification, they can simply ignore it and it will remain as a notification until it is dismissed. New notifications from Face2Rec will not overwrite old ones due to each notification having its own notification ID. This allows the user to see their recent activity. **Figure** 10 shows how a notification may appear on an Android smartphone and **Figure 11** shows the same notification on Google Glass.

Figure 11. Google Glass notification. If Glass tapped, the option to scroll through the text is provided.

Figure 10. Android device notification.

RESULTS

To test OpenCV/JavaCV's algorithm accuracy, a group photo and a solo photo was used. In a group photo, Face2Rec scanned all the faces to find one who is in the database. If no one in the photo was in the database, then Face2Rec would display an "unknown" notification. If it found someone who is in the database, then it will calculate and display an acceptance level. During our group photo testing, the algorithm was able to pick out the individual who was in the database and provide an acceptance level. However, if the individual was far from the camera, then the individual's acceptance level may qualify the match as only potential. This could be because of the image quality being too low due to the distance. If the individual in the database uses glasses, then that can also confuse the algorithm. In one instance, the algorithm recognized one student author as the other student author potentially because they both wear glasses and the image was not a close-up. A solo photo had the same level of accuracy as a group photo. Another OpenCV and JavaCV issue was that it was unable to process large file sizes. Face2Rec can potentially crash due to an image being greater than a couple hundred Kilobytes. Fortunately, the Google Glass photos are small so Face2Rec does not crash. However, photos taken with the Android smartphone does crash the application due to their high quality. OpenCV and JavaCV also may not always perform accurate facial recognition when a head is tilted in a photo. Based on this information, OpenCV/JavaCV's facial recognition algorithm was not very accurate. It is a good introduction to the potentials of facial recognition, but due to the noted cases, it should not be relied upon. The application, Face2Rec, however, did perform as expected. Face2Rec was able to take in input from Google Glass and the smartphone's gallery and output results based off OpenCV/JavaCV's algorithm calculations. The Google Glass took a few seconds, as great as ten seconds during some test runs, to send photos to

the Android smartphone. This means Face2Rec did not work as quickly as it should. However, since Google Glass Photo Sync was used, the speed to send photos is out of our control.

DISCUSSION

The overall purpose of this project was to create an Android application that communicated with Google Glass and performed facial recognition on images. Face2Rec successfully executed all our objectives for this project. Face2Rec is able to identify the two student authors and can send notifications to all devices. However, while the application itself is able to perform all our goals, it is hindered by OpenCV/JavaCV's unreliable algorithm and by the Google Glass slowness.

Future Updates

A future version of Face2Rec may involve a custom function to send photos to the Android phone faster. Other algorithms besides OpenCV/JavaCV need to be used that can handle all the variables as noted in our *Results* section. Face2Rec also requires an update in order for non-programmers to easily update the database with their persons of interest. At this time, the user needs to use Android Studio to manually input more people into the Face2Rec database. Lastly, Face2Rec can be improved by incorporating a web crawler that can search online photos and pull information about individuals once it finds a match.

CONCLUSIONS

Face2Rec is a good starting point into what could be a highly useful application. After working on it for only ten weeks, it progressed quickly and has a lot of potential. If Face2Rec is improved upon as noted in our *Discussion* section, then it can make the world a safer place. At this time, the facial recognition algorithm used in Face2Rec is not 100% accurate. Therefore, it should not be officially used by law enforcement, the government, or any other institution. However, facial recognition is being continuously worked on by multiple companies so an accurate algorithm is expected one day. The code of this project is open source and can be used by anyone in order to improve it. The project was saved in multiple parts, but the combined final version of the code can be found on GitHub.[35]

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ABOUT THE STUDENT AUTHORS

Gabriella A. Mayorga and Xuan Do completed this project while earning a Bachelor of Arts in Computing and Informatics from Rowan University. They both plan to graduate in the Spring of 2019. They were selected by Dr. Heydari to participate in Rowan University's Summer Undergraduate Research Program (SURP) in 2018 which provided their funding for 10 weeks to develop this application. Gabriella A. Mayorga plans to focus on cybersecurity and is interested in software development and mobile applications. Xuan Do plans to pursue her career in web development.

PRESS SUMMARY

Using facial recognition and detection to identify people is a useful tool for law enforcement, the government, and universities. Combining that technology with smart glasses gives those agencies the ability to identify people in a hands-free manner. Our project, Face2Rec, is designed to do that by using an Android smartphone that communicates with Google Glass. Face2Rec searches for faces in a Google Glass photo and if a face is found, it compares those faces against a user-created database of people. The user is informed if there is no face detected, no match found, partial match found, or match found. If there is a match, the user is given additional information. All results are displayed on their Android smartphone and smart glass. The user is also given an acceptance level for each result to independently determine the reliability of the information.

Sexual Assault Among College Students Attending a Historically Black College/University

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ABSTRACT

Sexual assault constitutes a significant public health problem on college campuses including historically Black colleges and universities (HBCU). Recent research suggests that sexual assault is increasing on college campuses. However, there are few studies examining the prevalence and risk factors for sexual assault at HBCUs. To address this gap, the current study examined the prevalence, correlates, and outcomes of sexual assault at an HBCU. Participants in the study were 264 undergraduate students from an HBCU in the mid-Atlantic region. The majority of participants were female (71%), African American (91%), and seniors (41%). After providing informed consent, participants completed a Climate Assessment survey administered by the university's Office of Diversity. Findings revealed that since starting college about 20% of students experienced sexual contact without consent. Of those sexually assaulted, 20% reported they were incapacitated or under the influence of alcohol (15%) at the time of the assault. About 17% of those assaulted experienced a physical injury and/or poor mental health outcomes (e.g., anxiety, depression, flashbacks). Participants reported not disclosing information of their assault due to embarrassment, afraid of retaliation from the perpetrator, believing it was a private matter. Close friends were more likely to be told about sexual assault. The study supports the need to address sexual assault on HBCU campuses through strong prevention and intervention programs and to address barriers to reporting.

KEYWORDS

Sexual Assault; HBCU; College Students; Prevalence; Risk Factors; Outcomes; Barriers of Reporting; Sexual Assault Climate Assessment Survey

INTRODUCTION

Sexual assault is defined as any sexual acts committed against someone without that person's freely given consent.[1] It can be categorized as sexual activities such as incest, molestation, fondling, or forced sexual intercourse.[2] Women between the ages of 18 and 24 are at a higher risk of experiencing sexual assault.[3] Sexual assault remains a problem on college campuses, particularly among women.[4] Unfortunately, most studies examining sexual assault focus on predominately white (PWI) college campuses and not minority-serving institutions. This paper reviews the extant literature on sexual assault, general findings on studies of sexual assault on college campuses, and a review of findings from those few studies that examine sexual assault at HBCUs.

Sexual Assault in the U.S.

According to the Rape, Abuse, and Incest National Network (RAINN, 2016), every 107 seconds, an American is sexually assaulted.[3] The U.S. Department of Justice's National Crime Victimization Survey (as cited in RAINN, 2016) reported there is an average of 293,066 victims (age 12 or older) of rape and sexual assault each year.[3] Approximately 19.3% of women have experienced rape and 43.9% experienced other forms of sexual assault-including unwanted sexual contact (*i.e.,* kissing or touching) or sexual coercion.[5] About 3% of American men or 1 in 33 have experienced attempted or completed rape in their lifetime. 2 out of 3 sexual assault incidents are not reported to the police.[3] The LGBTQ community is also affected by sexual assault; 46.4% lesbians, 74.9% bisexual women and 43.3% heterosexual women have experienced sexual violence other than rape during their lifetime.[6] Cumulatively, these findings suggest that sexual assault is a problem in the U.S. and that there is a need to explore and better understand risk factors for sexual assault among at-risk populations, such as college students.

Sexual Assault on College Campuses

College and university administrators, faculty, staff, and students are aware of campus sexual assault, yet it remains a significant problem on college campuses. One in five women and one in 16 men are sexually assaulted while in college.[7] A study done by Jordan, Combs, and Smith (2014) examining sexual assault and academic performance found that more than 40% of the female participants had experienced rape or sexual assault during their teen years.[8] 24% of women experienced sexual victimization

during their first semester of college in addition to another 20% during their second semester of college. It is common that college-aged female victims know their offender in about 80% of rape and sexual assault victimization.[9] Although women are more likely to be victims of sexual assault, a Campus Sexual Assault study (CSA) found that approximately 6.1% (n = 84) of males reported experiencing attempted or completed sexual assault since entering college. Half of them (n = 50, 3.7%) experienced a completed sexual assault. Incapacitation of the victim is more prevalent among women. Incapacitation involves the use of alcohol or drug use, being passed out, asleep, or being unable to consent regardless of the perpetrator being responsible or the victim's own actions to participate in substance use.[10] 7.8% of women (n=466) were victims of alcohol or drug enabled sexual assault. 0.6% of women (n=31) were victims of confirmed drug-facilitated sexual assault while 1.7% (n=103) were victims of suspected drug-facilitated sexual assault. 1.0% (n=48) were unable to provide consent. According to the College Alcohol Study (CAS), 3.4% of women who were raped were unable to provide consent due to incapacitation and 3.1% experienced drug-facilitated sexual assault (DFSA).[10]

Sexual assault is highly underreported. Evidence shows victims are more likely to disclose to friends and less likely to formally report the incident to authorities. For instance, a study found that in 2/3rds of incidents, the victim disclosed to another person, usually a friend, but not family or school officials.[11] Sable, Danis, Mauzy, and Gallagher (2006) described psychological and legal barriers that have discouraged college students who are victims of sexual assault from reporting.[12] This study identified 13 barriers to reporting for women and 14 for men. Each characterized which barrier were reasons to not report by importance. Women's barriers to reporting included fear of retaliation by the perpetrator, financial dependence, did not want family or friend to be prosecuted, and lack of resources to obtain help. Men's barriers to reporting included shame, embarrassment, confidentiality concerns, and fear of not being believed. Many women often may not report because they know the perpetrator. Men are less likely to report due to masculinity stereotypes. Alcohol and drug use are another factors that explains college students not reporting to law enforcement.[13] Self-blame typically occurs when the victim was under the influence.[14] Alcohol seems to be a key contributor in campus sexual assault.[15] Typically, both the perpetrator and the victim had consumed alcohol. Abbey, McAuslan, and Ross (1998) reported that 42% consumed alcohol prior to the incident and 53.4% reported that the perpetrator had consumed alcohol prior to the incident.[16] Alcohol effects on motors skills may limit women's ability to resist sexual assault attempts. It also affects perceptions of responsibility. Alcohol consumption among men is perceived to be justification for inappropriate behavior allowing them to feel comfortable when engaging in such risky behaviors. However, some women who consume alcohol may take the blame for sexual assault.[15] These findings suggest that sexual assault is a problem on college campuses and must be addressed. Moreover, most of the research reviewed has focused on predominately White college campuses. While it is important to study sexual assault regardless of the type of campus, there is a need to examine the prevalence and outcomes of sexual assault on all college campuses, including Historically Black Colleges and Universities (HBCU).

Sexual Assault at HBCUs
As cited above, there is an extensive amount of research focusing on sexual assault targeting students at Predominately White Institutions (PWI) however, limited research exists addressing sexual assault among students attending minority-serving institutions such as Historically Black Colleges and Universities (HBCU).[17] Research suggests that there may be differences in sexual assault rates between these institutions. Differences between African American women at non-HBCUs to those at HBCUs is unknown.[18] According to Lindquist et al. (2013), approximately 9% of undergraduate women had experienced physically forced sexual assault prior to entering college. 3.4% experienced incapacitated sexual assault and 2.4% experienced both prior and since entering college.[18]

The goal of the HBCU- Campus Sexual Assault (HBCU-CSA) study was to document information on prevalence, consequences, and risk factors of sexual assault among African American college students.[19] This study included 3,951 women from four HBCUs. 14.9% reported experiencing attempted or completed sexual assault prior to entering college. 14.2% reported experiencing attempted or completed sexual assault since beginning college. 3.8% of women experience physically forced rape, and 4.8% experienced rape while incapacitated and unable to provide consent. Prevalence estimates of victims suggest that some were incapacitated and unable to provide consent. 4.0% of women were victims of alcohol or other drugs (AOD) which includes the victim voluntarily consuming alcohol prior to the incident and 0.3% were victims of DFSA. Rates of sexual assault when the victim was incapacitated and unable to provide consent appears to be considerably lower for women at HBCUs than non-HBCUs.

The HBCU-CSA study also examined risk factors among victims of sexual assault at an HBCU. Prior victimization is associated with experiencing sexual assault after entering college. Women dating at least one person after entering college compared to those not dating were more likely to experience any type of sexual assault. Compared to women with no male sexual partners, women who had between one and five male sexual partners since entering college were more likely to experience incapacitated sexual assault and those with more than five male sexual partners were more likely to experience all types of sexual assault. Women who reported getting drunk were likely to be victims of incapacitated sexual assault. The study described individual and behavioral risk

factors associated with sexual assault. The type of sexual assault was associated with a given risk factor. Factors such as prior victimization and party attendance were associated with physically forced sexual assault. Behavioral influences such as substance use and depression were associated with incapacitated sexual assault. Victims of incapacitated sexual assault reported engaging in alcohol or drugs prior to the incident. Other risk factors such as accepting drinks from strangers or sorority membership were associated with both forced and incapacitated sexual assault since entering college.

Women victimized by sexual assault were found to have significantly more symptoms of depression and were more likely to experience post-traumatic stress disorder (PTSD) compared to women who had not been sexually assaulted.[18] Sexual assault victims were more likely to report the incident to family, friends, law enforcement, or crisis centers. Victims were also more likely to drop their classes, change majors, or seek counseling.

Given the limited amount of literature to address sexual assault on an HBCU campus, the current study attempted to answer the following questions:

1. What is the prevalence of sexual assault on an HBCU campus?
2. What are the correlates associated with sexual assault at an HBCU?
3. What are the outcomes associated with sexual assault at an HBCU?
4. What are the reporting practices and barriers to reporting sexual assault at an HBCU?

METHODS AND PROCEDURES

Participants

Participants were 264 undergraduate students attending a co-ed HBCU located in the northeast region of the US. Among the participants, 71.7% were female and 28.9% male. A majority of participants were African American (90.9%), 4.3% were Caucasian, 4.2% were Hispanic or Latino, 3% were American Indian or Alaska Native., 2.7% were Asian and 1.1% Native Hawaiian or Pacific Islander. Most participants were seniors (40.9%), 22% were juniors, 21.2% were freshmen and 15.2% were sophomores. Participant ages ranged between18-24. 11% (n=29) were 18 years of age, 16.3% (n=43) were 19, 15.2% (n=40) were 20, 17.8% (n=47) were 21, 11.7% (n=31) were 22, 9.8% (n=26) were 23 and 18.2% (n=48) were 24 or older. 79.2% were heterosexual, and 2.7% were gay. Approximately 39.2% reported living in a college residence hall and 4.6% living on-campus. 94.3% of participants were full-time students and 5.3% were part-time.

Procedures

The university's Office of Diversity submitted and received IRB approval to administer a campus-wide Climate Assessment Survey developed by an educational technology company which offers online training on various topics including sexual assault prevention. The survey instrument is based on a model template provided by the White House Task Force Report to Protect Students from Sexual Assault[20]. The survey was administered during the 2015-2016 academic year. Permission to assist with recruitment, data collection, and to utilize data from the study was granted to the student investigators from the Office of Diversity. All undergraduate students were recruited via email which included a hyperlink to the online questionnaire. Participants were also recruited via convenience sampling, flyers on social media. The online questionnaire remained available from December 2015 to February 2016. The home page of the study included a description of the study and allowed students to indicate their consent by clicking yes or no to proceed. The participants' identities remained anonymous throughout the survey and they were given the option to withdraw-or skip questions at any time without penalty. To ensure confidentiality and anonymity, participants were given a link to a site that would delete their web browsing history after completing the survey. The online survey was approximately 30 -45 minutes. After completion of the survey, on and off campus resources were provided for mental health services and sexual assault/relationship violence support in case of any discomfort. Participants were then directed to another link to participate in a raffle to win a notebook computer. All participants were thanked for their participation.

Measure

A web-based Campus Climate Survey was developed by an educational consulting company and aimed to identify the prevalence, perceptions of campus policies and reporting, risk factors, and outcomes of sexual assault on campus. The climate survey consisted of 14 sections with a total of 101 questions. For the purposes of this study, only three sections were analyzed:

1. Demographics: This section asked background information such as biological sex, race, classification, living arrangements, and enrollment status.

2. Alcohol and Drug Use: This section asked participants about their experiences with alcohol and drugs. Drinking or other under the influence at the time of the incident. A Sample item include "since the beginning of this school year, about how often have you consumed alcohol?" Participants responded to the frequency in

which they engaged in a behavior from 1=Never, 2=Less than once month/A few times, 3=Once or twice a month, 4=Once or twice a week, and 5= Daily or almost daily.

3. Sexual Violence: This section asked questions about the nonconsensual sexual contact which targeted five types of sexual contact (oral sex, touching of a sexual nature, sexual intercourse, anal sex and sexual penetration with a finger or object). Sample items include, "have you experienced sexual contact without your consent since you became a student or just prior to the incident." In addition, items measured outcomes and barriers of reporting such as "did the incident have a negative impact on the following "(*i.e.,* schoolwork, social activities, or social relationships). Items were measured using yes/no responses, and multiple response options (i.e., check all that apply).

Study analyses

This study is a descriptive study that captures information involving unwanted sexual experiences of students. Descriptive analyses were conducted to identify participants' demographics, prevalence rates, risk factors, and barriers to reporting associated with sexual assault and the outcomes of victimizations. Chi-square analysis was done to determine the prevalence rates of sexual assault by gender and classification.

RESULTS

Prevalence of Sexual Assault at an HBCU

Prevalence estimates of sexual assault prior to and since entering college was determined. Past research suggests that experience of sexual assault prior to college is a correlate of sexual assault occurring during college. Of the 264 participants, completing the survey, 174 responded to questions about sexual assault. Of these students, 25.3% (n=44) reported having experienced sexual contact without consent before becoming a college student. 20.6% (n=34) reported experiencing sexual contact without their consent since becoming a student. 17.6% (n=6) reported experiencing completed sexual contact through physical force or threats prior to entering college, 20.6% (n=7) reported after the start of the 2015 academic year and 11.8% (n=4) said both before and since the start of the academic year. 14.3% (n=5) had experienced attempted sexual contact by physical force or threats, 11.4% (n=4) after the start of the academic year and 8.6% (n=3) both before and after the academic year. As shown in Table 1, women were significantly more likely to report sexual assault compared to men; x^2 (1) =9.47, p<.01. Table 2 shows that upperclassmen were more likely to report being sexually assaulted compared to lowerclassmen; x^2(1) =3.99, p< .05. Since becoming a student, 20.2% reported experiencing verbal or non-physical coercion and a smaller percentage reported attempted verbal or non-physical coercion (11.4%). Since becoming a student, 6.1% suspected that someone had sexual contact with them when they were unable to provide consent or incapacitated, and 9.1% had experienced someone having sexual contact with then while being unable to provide consent or incapacitated.

Responses	Female		Male	
	N	%	N	%
Yes	31	26	3	6
No	74	89	50	94

Table 1. Prevalence of sexual assault since becoming a student by gender. 21 students did not report their gender.
x^2 (1) =9.47, p<.01

Classification	Yes		No	
	N	%	N	%
Lower Classmen	7	12	54	88
Upper Classmen	27	24	85	76

Table 2. Prevalence of sexual assault since becoming a student by classification. Lower classmen represent freshman and sophomores. Upperclassmen represent juniors and seniors.
x^2(1) =3.99, p< .05

Correlates Associated with Sexual Assault

Of the 34 participants who reported experiencing sexual assault since becoming a student, as shown in Figure 1, 5 participants (15%) had been drinking alcohol just prior to the incident, 3 participants (8%) were drunk, and 7 participants (20%) had been incapacitated and not able to give consent or stop what was happening. Participants were also asked who had the unwanted sexual contact with them. 10.2% said it was a male and 1.1% female. Victims also reported whether the incident involved the other person's use of alcohol (44.8%) or drugs (83.3%), and their own use of alcohol (20.7%) or drugs (96.7%). From these results, alcohol consumption and drug use are a contributing factor to sexual assault.
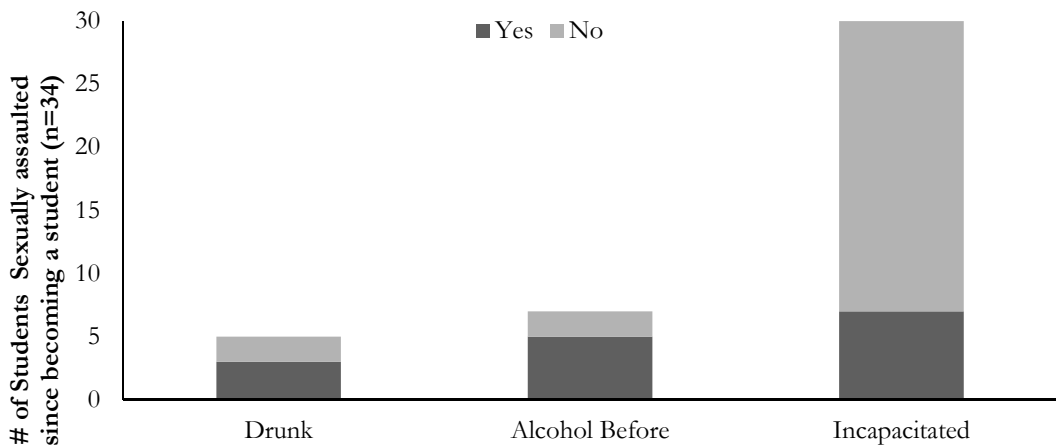
Figure 1. Alcohol-invloved correlates associated with sexual assault

Outcomes Associated with Sexual Assault

17.2% of victims reported physical injury after the incident. Many of them faced sexual trauma outcomes presented in **Figure 2**. The highest reported was anxiety (70.4%) and the lowest was alcohol or substance use (15.4%). As a result of the incident, victims reported being behind in their schoolwork, performing poorly on assignments, or missing a class. The incident also had a negative impact on their jobs, social activities, and intimate relationships. 22.2% sought medical treatment after the incident, 25.9% sought counseling services and 7.4% sought advocacy services.
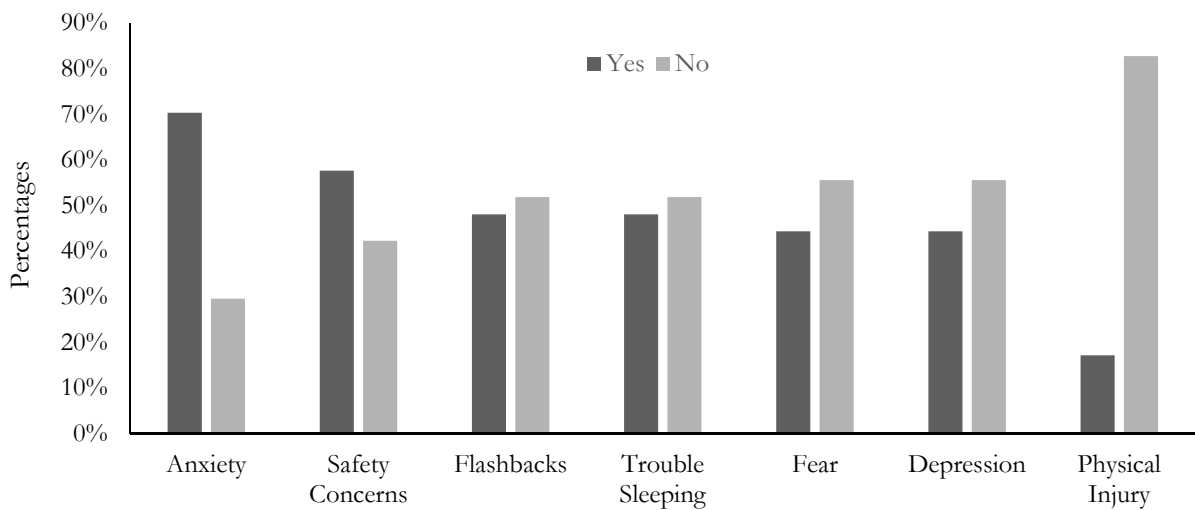
Figure 2. Reported outcomes associated with victims of sexual assault.

Barriers of Reporting Sexual Assault

Approximately 22% of the sample reported a lack of knowledge of where to get help regarding sexual assault resources on campus. 24 % did not understand how to report a sexual assault on campus. Victims were asked who they told about the incident of sexual assault as shown in **Figure 3.** The majority had told a close friend other than a roommate or no one and were least likely to report to police or campus faculty. Victims were also asked why the chose not to report their assault as presented in **Figure 4**. Answers varied from embarrassment, wanting to deal with it on their own, afraid of retaliation, didn't want the offender to get in trouble, or afraid of not being believed.
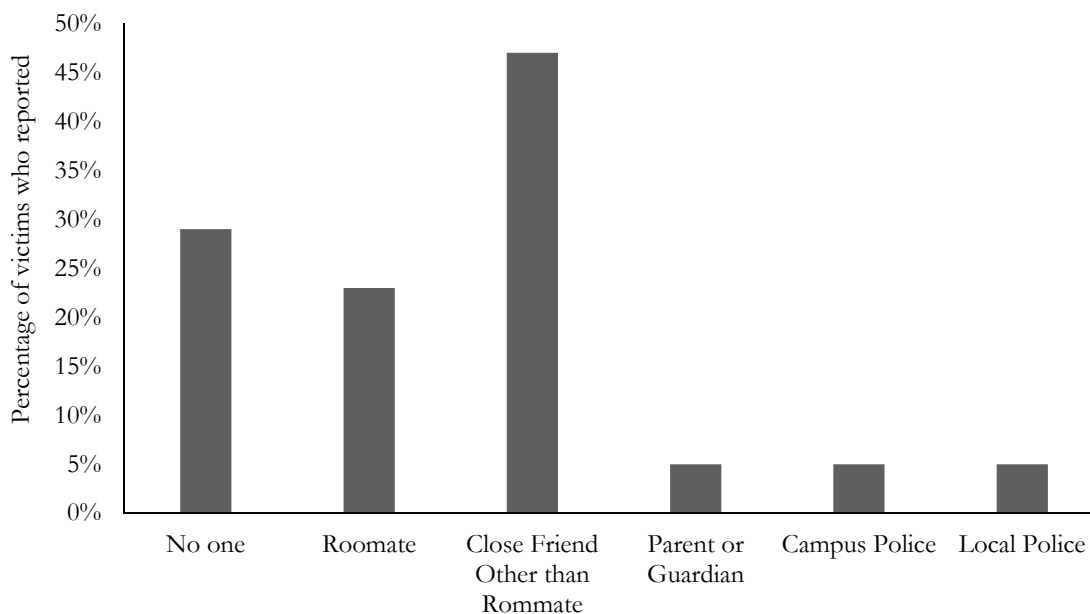
Figure 3. Reporting of sexual assault. Percentages are represented by those who reported being sexually assaulted.

Figure 4. Percentages are represented by those who reported being sexually assaulted.

DISCUSSION

The purpose of the study was to identify prevalence rates, correlates, outcomes, and barriers to reporting sexual assault at an HBCU. Our results suggest that since starting college about 20% of student surveyed experienced sexual contact without consent. This finding is similar to previous research studies examining sexual assault among college students. For example, similar to Krebs *et al.* (2007) and Sinozich & Langton (2014) 20% of undergraduate women attending a PWI reported experiencing sexual assault

since entering college.[9,10] Also, consistent with previous research suggesting an association between alcohol use and sexual assault (Abbey, 2002).[15] our findings indicate that alcohol use does play a key role in sexual assault. Alcohol was the leading correlate associated with sexual assault which is consistent with the HBCU-CSA findings suggesting that women who reported getting drunk had higher chances of being victims of incapacitated sexual assault.[19] Another correlate was prior victimization. It is more likely for victims of prior victimization to experience sexual assault later in their lifetime. This is also consistent with findings from the HBCU-CSA study reporting that being a victim of forced sexual assault before college was significantly associated with being a victim of forced sexual assault.[19] Although a few men did report an occurrence of sexual assault, women were more likely to be sexually assaulted than men. Research suggests that there are barriers to reporting sexual assault and victims are more likely to report to friends or roommates. In addition, participants in this study indicated several reasons to not report which are comparable to findings from a study on barriers to reporting sexual assault.[12, 21] Participant's reasons for not reporting include but are not limited to embarrassment, did not want family or friends to know, or being afraid of retaliation. Other reasons include lack of knowledge of how or where to report incidents of sexual assault. Victims were more likely to experience safety concerns and anxiety after sexual assault. Also, concerns with trusting the college administration to protect victims and follow the necessary steps of reporting was a barrier.

While not examined in the current study, one theoretical framework that may aid in understanding these findings is the Intersectionality Theory. This theory posits that "people are often disadvantaged by multiple sources of oppression: their race, class, gender identity, sexual orientation, religion, and other identity markers. Intersectionality recognizes that identity markers (e.g. "female" and "black") do not exist independently of each other, and that each informs the others, often creating a complex convergence of oppression".[22, 23] Thus, the dominant group (e.g., men or campus officials) may be perceived as using power to normalize oppression within marginalized groups (e.g., women). In the case of sexual assault, women may be more likely to experience deleterious outcomes associated with assault, such as anxiety and safety concerns, due in part to not getting the type of support needed to address the assault for fear of not being believed by the current power structures or may not report for the same reasons (e.g., fear of retaliation and not trusting college administration).

Limitations
There are several limitations to this study. Given that this study was only done on one HBCU and the sample size was small, we cannot generalize the findings to other HBCU campuses. The response rate of the survey only represented 4% of the total student population.at the participating university. Also, the majority of participants were seniors which limit a representation of lowerclassman (particularly freshman) who are more likely to be victims of sexual assault. Since this was a self-report survey, we had to assume the accuracy of answers pertaining to sexual history as well as response bias. There was also missing data. This could have resulted from participant's option to skip questions, discomfort from answering the questions, or accurately recalling sexual history.

CONCLUSIONS
Sexual assault remains a problem on college campuses, especially among women. The significance of this study was to add to the existing body of literature by examining sexual assault at an HBCU. With the exception of the HBCU-CSA study, most past research addressing sexual assault primarily target PWIs, so this study aimed to eliminate that gap by adding to the extant research on sexual assault at HBCU's. Given that there is limited research on sexual assault in HBCU populations, future research could examine if there are differences among students attending a PWI and HBCU. This could allow researchers to determine if there are racial differences in sexual assault and its impact on students. Additional research should focus on other aspects of sexual assault such as consent and what prevention methods can be implemented to decrease and eventually eliminate sexual assault on college campuses. Addressing sexual consent is an integral part of understanding sexual assault. Within sexual consent, research could examine the role of alcohol in consenting to sexual activity. One key finding of this study was that alcohol was the only factor associated with sexual assault. Interventions should be created to help students reduce alcohol consumption while educating them about the consequences of drinking on their health and mental capacity. The results of this study also indicated the need for interventions to educate students about sexual assault and how to prevent it. Possible interventions could include self-defense courses and workshops that address sexual assault and prevention strategies. According to our study, a close friend other than a roommate was more likely to be told about the assault. Interventions should, therefore, target individuals who are told about incidents of sexual assault. Targeting these individuals may help better assist victims of sexual assault. Assistance could include helping the victim to report, provide them with resources such as counseling, medical examination facilities, or shelter if needed. It is important to make students feel comfortable about reporting to the police or other authority figures. Lastly, observations of university policies on sexual assault may address concerns of reporting. Students should learn as a freshman entering college where and who they can report incidents of sexual assault and be assured they will be treated fairly, attain proper help and safety.

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PRESS SUMMARY
This article examines sexual assault among African American college students by providing prevalence rates, correlates, outcomes, and barriers to reporting associated with sexual assault. Findings suggest that sexual assault remains a problem and is prevalent on an HBCU campus.

Examining Collection Biases Across Different Taxonomic Groups: Understanding How Biases Can Compare Across Herbarium Datasets

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ABSTRACT

Specimen-based data are an invaluable resource for an increasing diversity of scientific fields, including global change biology, ecology, evolution, and genetics; however, certain analyses of these data may be limited by the non-random nature of collecting activity. Geographic, temporal, and trait-based collecting biases may consequently affect the understanding of species' distributions, obviating the need to determine what biases exist and how they may impact further analyses. Trait-based biases were examined in herbarium specimen records of two abundant and diverse families (Asteraceae and Fabaceae) in a well-collected and digitized region (California) by comparing geographic-bias-adjusted simulations of random collections to actual collecting patterns. Collecting biases were fairly similar between families for a number of traits, such as a strong bias against collecting introduced species, while seasonal collecting biases showed a peak in activity in the Spring for both families. However, while there was only a dip in the fall for Asteraceae, Fabaceae were seriously under-collected for the majority of the year. These results demonstrate that significant collecting biases exist and may differ depending on the dataset, highlighting the importance of understanding the dataset and potentially accounting for its sampling limitations.

KEYWORDS

INTRODUCTION

Herbaria, collections of dried and pressed plant specimens, are excellent sources of botanical, ecological, environmental, and other data, helping us understand the changes species, and even ecosystems, have experienced over time. Herbarium specimens have enabled studies of species distributions, pollution, climate change, and even disease spread.[1, 2] The digitization of herbarium specimens has provided researchers with large datasets that are easily accessible; the median number of specimens being used in papers is notably higher when scientists use digitized databases.[1] However, despite all the benefits they provide, herbarium specimen data may contain biases, since collectors rarely collect specimens in a truly random fashion, resulting in over- and under-collecting of certain types of species, in certain locations, or at certain times. Collectors were historically focused on the discovery of new species and their distribution in particular areas, and this non-random collection pattern has thus become problematic only recently. Indeed, only after researchers began to use these collections for studies other than taxonomy or distribution, did legitimate concerns about several types of collection biases start to arise since, as mentioned above, they can drastically affect the results of any studies based on herbarium specimens.[1-3]

For instance, there are geographical biases;[3-11] taxonomic biases that occur when certain types of plants are collected over others (*e.g.*, for a specific research project);[12, 13, 14] temporal biases[9, 10, 13, 15-19] phenological biases that occur based on when the plants flower;[20] and biases based on individual morphology of the plant such as a particularly tall or oddly-shaped specimen. As these biases have come to light and the purpose of herbaria evolved, increasing attention is being paid to how these biases occur and possible methods to correct or account for them.[3, 21-23] As we try to integrate these old collections into current studies, aside from taxonomy and distribution, it is becoming more clear that in order to achieve accurate results, we need to rethink the way we approach specimen collection. Historically, a sterile specimen without flowers or fruits would have not been considered worthwhile to collect because they could be nearly impossible to identify. So while herbaria are excellent reflections on reproductive phenology, there is little reference for vegetative traits.

Many of the studies listed above have identified biases in specimen-based datasets with limited taxonomic scopes (*i.e.*, using few species), while even fewer identify how these biases compare between different datasets.[21] In this study, trait-based biases were compared in datasets of two different taxonomic groups, the sunflower family (Asteraceae) and bean family (Fabaceae), in the U.S. state of California. These plant families are widespread in the state (There are around 1,400 known

Asteraceae species and 700 Fabaceae species present), and their morphological, geographical, and taxonomic diversity allow us to test for multiple trait-based biases. Furthermore, active herbarium specimen digitization in California has made large amounts of previously hard to access specimen data available, enabling more comprehensive analyses of biases on a larger scale. Understanding the similarities and differences in collecting biases shared among different institutions may inform future analysis using these data.

METHODS AND PROCEDURES

Specimen data for California Asteraceae and Fabaceae was downloaded using the iDigBio portal (idigbio.org). Data cleaning consisted of removing any erroneous (*e.g.*, non-plant, outside California) specimens, standardization of taxonomic names using the Taxonomic Name Resolution Service, and removing duplicate collections (*i.e.*, specimens of the same species collected in the same county on the same day). Records not classified to the species level and records of species with fewer than 50 specimens were excluded, as they were not considered to have enough specimen information to be accurately tested. The resulting dataset consisted of 151,035 specimens of 612 Asteraceae species and 78,744 specimens of 276 Fabaceae species.

Random datasets of Asteraceae and Fabaceae collections were simulated separately using a Monte Carlo approach similar to that of Schmidt-Lebuhn *et al.* 2013 that accounted for nonrandom spatial sampling (geographic biases). Briefly, simulated specimens of a given species from both families were "collected" at a frequency directly proportional to the level of collecting activity of actual specimens in the counties in which the species was collected in the actual dataset. Essentially, our simulated data sets imitated the levels of actual collection efforts, and we generated thousands of these simulations to mimic conditions if collection was truly a random effort. For each dataset (Asteraceae or Fabaceae), 10,000 simulated datasets were created. To compare the random simulations to the actual collections, the collection numbers from all simulations for a specific species were averaged together; this average was then compared to the observed number of specimens for the species in actual herbaria.

Data for growth habit (herb, shrub, or tree), generation time (annual or perennial), plant height, nativity (introduced or native), flowering period (greater than 3 months), and elevation (less than 100 meters) of each species were determined using the Flora of North America website (efloras.org), the USDA PLANTS database, Calflora.org, and other reputable sources. It should be noted that for growth habit tests, due to the relatively low number of tree species we examined in the Fabaceae family, we combined shrubs and trees into one group, woody plants. This group was then compared to Asteraceae shrub species. We also determined which species flower each month of the year for both families, and used this data to determine if there was a collection bias towards certain months. The number of all species collected in a particular month was averaged (total number of specimens divided by number of species). This means there is some overlap in the data as numerous plants flower longer than one month.

The percent difference was calculated between the average number of specimens generated by the simulation within the group with a given trait to the actual number of specimens with the trait in the actual dataset. A negative percent difference indicated that the bias exists against specimens with the trait, and a positive percent difference indicates that a bias exists towards specimens with the trait. The simulations for each species were used to create random expectations in the form of histograms. We then compared the simulated expectations to relative to the number of actual specimens in the database (see the dashed line in Figure 1).

RESULTS

As expected, the species tested exhibited a variety of traits. This made it possible to test for multiple biases and to compare biases between families.

Both native Asteraceae and Fabaceae species were over-collected relative to an expectation of random collection. Specimens of native Asteraceae were ten percent more abundant in the actual dataset than the simulated dataset (**Figure 1**), and specimens of native Fabaceae were nine percent more abundant. In contrast, introduced species were under-collected by 23% in Asteraceae and 75% in Fabaceae.

For the growth habits of specimens, forb/herb species in both families were over-collected (Asteraceae: five percent; Fabaceae: ten percent). In comparison, shrub specimens in Asteraceae were over-collected by 11%, while woody specimens in Fabaceae were under-collected by 32%.

The pereniality of species was examined, and while annuals were over-collected by nine percent in Asteraceae and under-collected by 14% in Fabaceae, perennials were over-collected in both Asteraceae and Fabaceae by ten percent and 14% respectively.
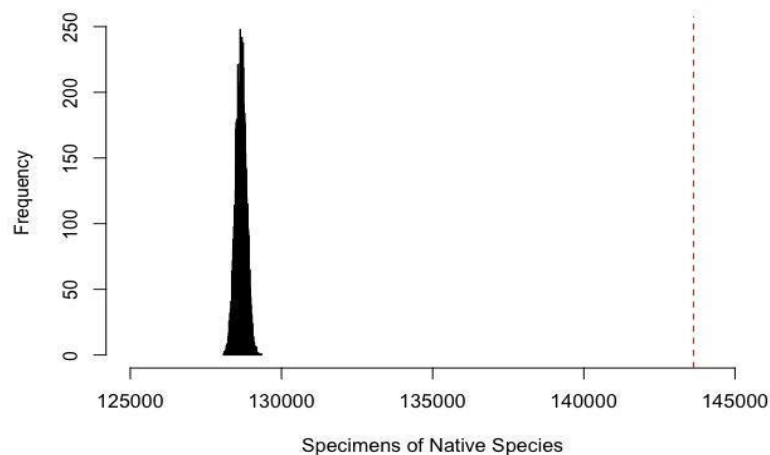
Figure 1. Histogram demonstrating the bias toward collecting native species of Asteraceae in California. The distribution on the left represents the number of simulated datasets with a given number of specimens with this trait, while the red dashed line on the right indicates the average of the actual collected specimens.

In addition, species with a maximum elevation of 100 m or less were also found to be over-collected in both families, with a percent difference of 22% in Asteraceae and 14% in Fabaceae.

In contrast, species with a flowering period of more than three months were considered, and these species were found to be over-collected by 14% in Asteraceae, but seriously under-collected by 30% in Fabaceae (**Table 1; Figure 2**).

Trait	Family	# of Species	Actual Value	Simulated Value	Percent Difference
Native	AST	547	143,630	128,662	11
	FAB	245	73,006	66,497	9
Introduced	AST	50	13,680	17,309	-23
	FAB	30	5,426	11,930	-75
Wooded	AST	89	22,592	20,221	11
	FAB	43	9,270	2,750	-32
Forb/Herb	AST	406	107,875	102,413	5
	FAB	233	69,474	65,996	10
Elevation <100 m	AST	285	92,099	73,615	22
	FAB	152	58,693	51,0304	14
Flowering >3 months	AST	292	85,577	74,158	14
	FAB	70	16,051	21,753	-30
Annual	AST	209	63,094	57,571	9
	FAB	82	24,699	28,356	-14
Perennial	AST	343	83,327	75,383	10
	FAB	170	49,212	42,827	14

Table 1. Results for test of collecting bias in specimens of Asteraceae and Fabaceae in California. Table shows trait tested, number of species for each trait, actual number of specimens, number of specimens expected if collecting effort lacked bias, and percent difference between actual and simulated number of specimens.
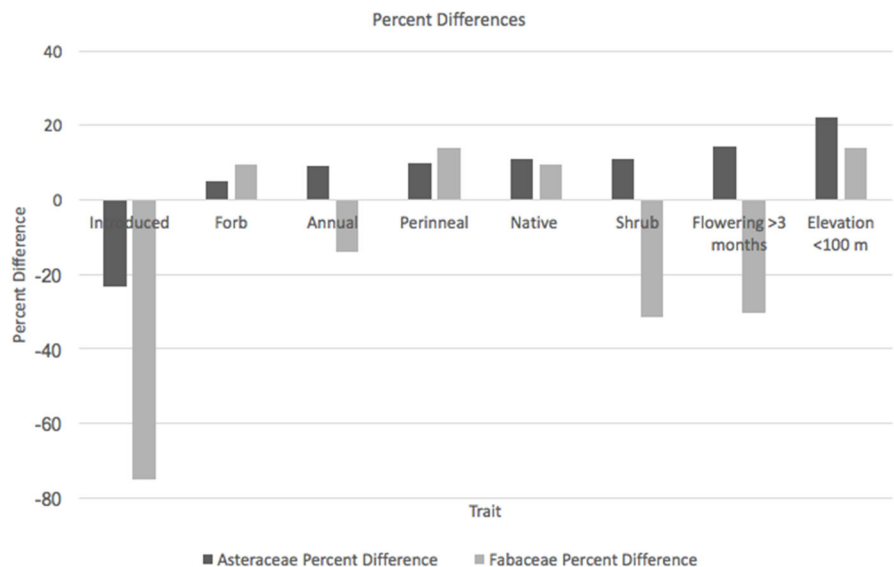
Figure 2: Percent difference totals.
This bar plot shows the calculated percent difference values compared to each other. Here you can see how collecting biases vary between taxonomic groups. While each family does exhibit the same types of biases, in many cases it is under-collected in one family and over-collected in the other.

When comparing the number of overall specimens collected each month (Figure 3), we found each family had a distinct trend. While species were over-collected during every month in Asteraceae, only April and May flowering species were over-collected in Fabaceae; all the other months showed under-collection. Asteraceae specimens were less over-collected during the late summer and early fall months (Aug-Nov) and were most over-collected in December and February.
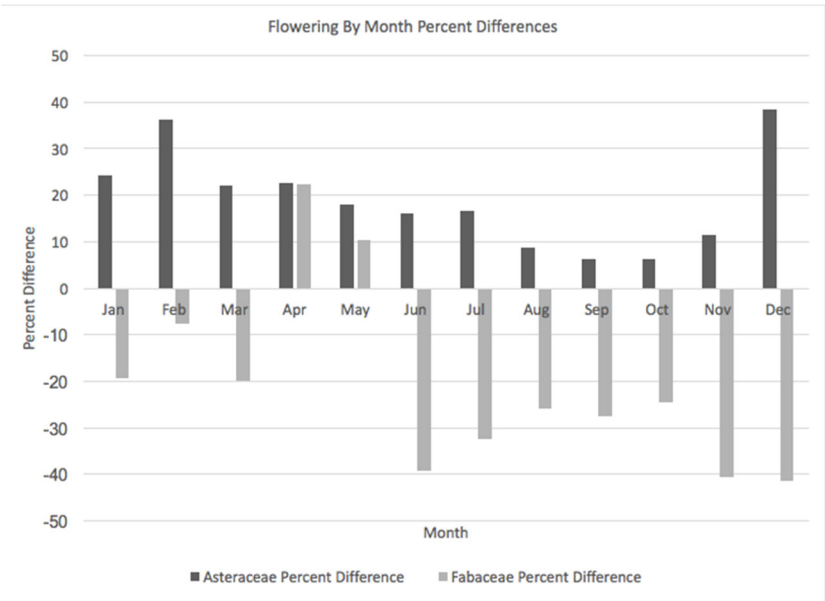
Figure 3. Percent Difference of specimens collected per month.
This graph shows the percent differences for each month of the year for California Asteraceae and Fabaceae species. Here you can see that Asteraceae experiences an overall over-collection bias, while Fabaceae experiences serious under-collection bias.

DISCUSSION

In the dataset of Asteraceae and Fabaceae herbarium specimens from California, significant biases towards collecting native, annual, perennial, forb, and shrub species were discovered. The results are also comparable to those of Daru *et. al* (2017), who conducted similar tests on vascular plants from Australia, South Africa, and New England. They used the same idea of comparing percent differences between actual collections and sets of randomly simulated datasets. However, unlike our comparisons between families, they tested differences within one family across multiple regions. It is important to consider, however, that at this point in time, not all herbaria have been digitized. The effort is still relatively new, and only a fraction of all existing collections are available online. This means that our dataset is not a perfect representation of species range, habitat, and traits. For the results discussed below it was assumed that the data well-represents the species studied.

It was predicted that introduced species would be over-collected, due to a stigma that the plants are more exotic or in high demand. However, this hypothesis was not supported, and introduced specimens in both families were under-collected more than was expected by chance (23% Asteraceae; 75% Fabaceae). This indicates that collectors are more likely to choose to collect native species, likely they seem more critical to study or have proven more useful in the types of studies herbarium data is typically used for. Alternatively, introduced species may be less abundant, especially at range edges. Historically, their distributions started much smaller and there was not much value in collecting them at the time. These conclusions are further supported by the evidence of over-collection of native species (11% Asteraceae; 9% Fabaceae). At their foundation, herbariums were mainly used for taxonomic purposes so it makes sense that collectors would focus on collecting the native plants for identification. The bias may be so pronounced in Fabaceae due to the fact that the sample size was small; compared to Asteraceae, and even small deviations from the mean would appear as high percent differences. Alternatively, the technique used to create the randomized collection data may be at fault. To determine how many specimens from each species should be collected for the unbiased randomized results, the number of counties the species was found in was considered; however, this did not account for how abundant the species actually were within the counties or the size of the county itself. For example, a species that appears in many counties may only be found a narrow range of disturbed habitats within those counties.

We also examined possible collection biases in species' growth habits. Plants classified as forb/herb were predicted to be collected more than shrubs because forb/herb species may be easier to collect, lacking woody structures. While the results supported the over-collection of forb/herbs in both families (five percent Asteraceae, ten percent Fabaceae), shrubs, too, were over-collected in Asteraceae by 11%. This inconsistency suggests that this pattern is ungeneralizable among taxonomic groups. However, the dataset of Fabaceae herb species was nearly five times as large as the woody species dataset (233 versus 43 species), meaning it could have produced an artificially large percent difference value. To avoid similar error in the future, it may be beneficial to compare families of the same size to make comparisons more accurate.

Looking at the elevation of species, it was predicted that specimens with a minimum elevation below 100 meters would be over-collected because they are easier for collectors to access. As expected, the simulations indicated an over-collection bias in both families. While 100 meters is not necessarily a very high altitude, these results suggest that as altitude increases, it gets harder to collect. In the future it would be beneficial to compare alpine species to sea level species to see if the trend remains.

Comparing annual versus perennial species, annuals appear typically more weed-like and may seem less desirable to collectors compared to the showier perennial plants. As such, annuals were expected to be more under-collected compared to perennials. In Asteraceae, annual species were over-collected by nine percent while perennials were over-collected by ten percent; for Fabaceae, annuals were under-collected by 14% while perennials were over-collected by 14%. In both families, there was a greater collection of perennials compared to annuals. It is a small percent difference in Asteraceae, however, which may indicate that the Fabaceae dataset was much smaller and more influenced by small deviations. Daru *et. al* 2017 also found a similar trend, with annual species being over-collected in both South Africa and New England.

It was predicted that species that flower longer than three months would be collected more frequently due to their availability. Collectors are more likely to collect specimens while they are in bloom, and species with a longer flowering period may thus be collected more often than a species that flowers for shorter periods. The results were mixed, as Asteraceae was over-collected by 14%, but Fabaceae specimens were under-collected by nearly 30%. This could be due to the availability of the families. Asteraceae is a more widespread family than Fabaceae, meaning that collectors might be over-collecting them compared to Fabaceae simply because there are more specimens and species to collect. This may also be due to flowering Asteraceae species standing out more in comparison to Fabaceae species, *i.e.*, with larger or more vibrant flower heads.

Finally, whether there was a bias toward collecting species that flowered in certain months of the year was examined. While there was a clear over-collecting bias in each month, it was much greater in some versus others (Figure 3). In Asteraceae, species that flower during the summer months are less over-collected than those that flower during winter months, which have percent

differences more than double those of the summer months. This could be due to conditions collectors are more likely to go out in. For instance, when the weather is neither excessively hot or extremely cold, collectors might be more willing to venture out and collect specimens. This could also be a result of the number of specimens that flower during certain months.

Alternatively, this could be a result of the availability of collectors. Over-collection of Asteraceae decreases drastically in August, September, and October, when most schools have begun again and many collectors may be teaching. Collection activity increases again in December, perhaps over Christmas Break when collectors may be able to go back out into the field. The number remains high in the spring months, likely due to the fact that at this time many species have begun to flower, making collectors more interested obtaining specimens.

For Fabaceae, the results were drastically different from the results of the Asteraceae test. As seen in Figure 4, there was a serious bias towards under-collection in almost every month of the year. Under-collection appears fairly constant throughout most months with the exception of two months in the spring, April and May, where there is an over-collection bias. This could be because of the availability of collectors during these months, as speculated for the California Asteraceae, or the result of the availability of the species. The peak seen in the spring could be due to spring bloom, as many species flower during this time and it is seen as a prime opportunity for collection. There is also notable under-collection during hotter months of the year, which could indicate collectors are more likely to go out when the weather conditions are more favorable. Daru *et. al* 2017 found a similar over-collection bias during the spring and summer months, which they conclude is due to collectors' desire to showcase species during these peak seasons. Due to the expense of collecting trips, it is logical to want to collect specimens that are blooming in order to best represent and identify the species. Much like how there was under-collection during hot months in California, there was serious under-collection in winter months in New Zealand, where winters are harsh and unfavorable. As hypothesized, they noticed that collection efforts increased during times of the year when schools are on vacation and during major holidays. However, they point out that tests like these fail to account for critical parts of a specimen's life cycle, such as buds and fruit maturation.[21] The more likely explanation is that the months in which collection efforts to showcase species in bloom occur line up with weather considered more agreeable.

In another recent study similar to ours, a number of additional different traits with collecting biases were highlighted and would be worth testing further in our dataset and other herbaria (Daru *et. al*, 2017). For instance, this study suggested an over-collection in roadside specimens and under-collection of threatened species, and also revealed that a large number of specimens were collected by a few major collectors, inferring that the individual biases the collectors held would influence the collections themselves. In future, it would be thus beneficial to see if these same types of biases exist within our study between families.

CONCLUSIONS
Our results demonstrate that there are collecting biases in herbarium data. Every test conducted supported a bias, either to under collect or over collect. This experiment was however, limited to studying the traits and species of just one state, California. The biases observed here may not be the same as in another state or country. These data do, however, serve as a good starting point for further, more in depth experiments to create actual correction methods for biases that can be used worldwide.

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PRESS SUMMARY

Biodiversity specimens are preserved organisms that can be used to study a variety of subjects, from the introduction of invasive species to climate change. Data about these specimens (*e.g.*, location, time, habitat) are aggregated in databases and used for analyses at larger scales than was previously possible. Many analyses assume that these data were randomly collected, which is important for accurate statistical results, but this is not always the case. Collectors are often biased in the way they collect specimens. This study examined how species' traits influence their collection and discovered that different collection biases exist in different groups of organisms. These results are important because the existence of collecting biases imply that trends determined from these data could be shaped by patterns of specimen collection rather than actual biological processes. Knowing what biases exist can help scientists understand how to account for them in their research.

Valveless Fluid Pumping *via* Zero-Net-Momentum Injection

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ABSTRACT

A novel mechanical method to valvelessly pump fluid has been developed using zero-net-momentum injection via a syringe and a tilted canister that function together as a periodic mass source and sink. Unlike previously discovered valveless pumping methods, this method does not require any elastic tubing and can be achieved by simple manual actuation, making it a simpler and less expensive valveless alternative. The flow rate is highly dependent on the frequency at which momentum is injected and retracted from the system. The direction of the flow can be changed by switching the location of the syringes. This pumping paradigm has potential applications in microfluidics where elastic channels are difficult to fabricate and valveless fluid actuation methods are preferred.

KEYWORDS

Valveless Pumping; Momentum Injection; Microfluidics; Fluid Mechanics; Windkessel Effect

INTRODUCTION

As lab-on-a-chip microfluidic devices proliferate for uses such as enzyme and DNA analysis and point-of-care medical diagnostics, the need arises for better valveless flow actuation methods.[1] Valveless fluid pumping in both open and closed loop systems is currently limited to four main methods: peristaltic,[2] impedance,[1] contraction pumping,[3] and dielectrophoresis (DEP).[4] Peristaltic pumping, fluid motion by compressions and expansions of an elastic tube a traveling wave motion, and impedance pumping, the transportation of fluid by tapping a section of elastic tubing in an otherwise inelastic tube repeatedly to drive flow, are both mechanical systems which require sections of elastic tubing in order to move the fluid. Contraction pumping, fluid flow created by multiple localized, rhythmic contractions of small sections of otherwise rigid tubing of differing lengths and amplitudes, has been seen in physiological systems such as the human duodenum and insects.[6] For micro- and nanofluidic devices, the smaller the scale, the harder and more expensive it is to manufacture sections of elastic or locally collapsible yet rigid materials, even as 3-D printing becomes increasingly ubiquitous.[5]

DEP is achieved by applying an electric field in the intended direction of fluid flow and does not require the elastic or locally collapsible materials needed for peristaltic, impedance or contraction pumping. Unfortunately, DEP has a number of other drawbacks, particularly if the material is not chemically inert. The fluid can undergo electrothermal flow from high temperatures and voltages. Electrolysis can also occur, which leads to gas evolution within the fluid, causing the pump to become drastically less efficient.[7] These effects can not only influence results but also limit what fluids can be pumped by DEP and the flow rates at which they can be pumped.

This paper presents a new paradigm for fluid pumping that does not require any elastic deformation of the membrane or an electric field applied to the fluid. Instead, fluid is pumped by introducing momentum into the system similar to the one introduced by Aboelkassem and Staples.[8-12] That pumping mechanism works by combining principles of impedance and contraction pumping to introduce momentum into the system using multiple localized, rhythmic contractions of elastic sections of an otherwise rigid tube. These elastic sections are actuated with a phase lag and are of different sizes and amplitudes which changes the otherwise symmetric hydraulic resistance in one direction and the fluid consequently takes the path of least resistance.

The pumping mechanism introduced in this paper generates a similar pattern of asymmetric hydraulic resistance without the need for any elastic or collapsible sections of tubing. Here fluid is inserted to and retracted from the system using a syringe and a partially tilted canister that function together as a periodic mass source and sink. The syringe is filled to the plunger while the canister is filled with the fluid as well as air. The syringe is actuated with a simple sinusoidal frequency but the tilt and air in the canister, hereby referred to as the Windkessel,[13] creates an asymmetry in the hydraulic resistance of the system which generates a net unidirectional

flow to be generated. This flow is generated despite the Windkessel remaining stationary throughout experimentation and zero-net-momentum being added to the system over one period of actuation. The actuation of the syringe can be done manually, which makes the paradigm potentially useful for inexpensive or emergency medical testing. This paper investigates and quantifies the effects that the actuation frequency of the zero-net-momentum injection has on the net flow rate in a single configuration as well as the paradigm's limitations.

METHODS AND PROCEDURES

To conduct this experiment, a closed, circular system of a constant diameter was created from a 1.5 m long piece of high-density polyethylene (HDPE) tube with an inner diameter of 6.35 mm and an outer diameter of 9.525 mm. Two 3.175 mm diameter holes were drilled into it 85.725 mm apart, in the axial direction for the syringe and Windkessel to be inserted. The ends of the tube were joined together by sliding another piece of HDPE tubing with an inner diameter of 9.525 mm over the ends of the primary tube, being sure to clamp it down using ties to prevent leaking. The tube was then completely filled with tap water, ensuring the removal of any air bubbles prior to experimentation.

Two identical 22 mL syringes were used. One was modified to make a Windkessel by removing the plunger. The syringe was filled to the plunger with 10 mL of tap water up with the top of the plunger attached to a Cool Muscle CM1-C-23L20C Servo Motor which allowed the syringe to be linearly actuated in a sinusoidal fashion at a set frequency. The Windkessel was filled with 4 mL of water and 18 mL of air at room temperature and atmospheric pressure. The tilt angle, θ, was held constant at approximately 5 degrees from the perpendicular as seen in **Figure 1**.
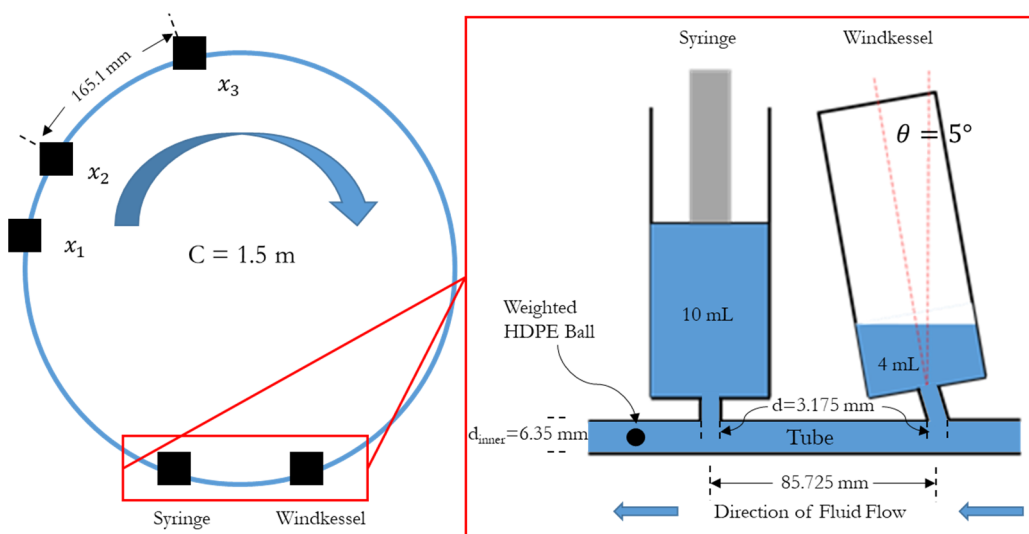
Figure 1. A generalized schematic of the experimental setup from a top view with an insert of the syringe and Windkessel from a front view. The circumference, C, the tilt angle, θ, and the most measurements have been noted as well as the direction of the fluid flow in the blue arrows. The x_i points indicate the set locations for the HDPE ball for measurements with x_1 being the start of actuation, x_2 being the start of timing, and x_3 being the end of timing.

To measure the net volumetric flow, Q, flowrate in the tube, knowledge was required about the time it takes to travel a known distance in the tube. An HDPE ball with a 3.175 mm diameter, weighted with a small metal pin to make it magnetic and as close to neutrally buoyant as possible, was inserted into the tube. It was then moved to x_1 at the start of each test using a magnet. The Servo Motor then actuated the syringe to begin the fluid flow, which reached its steady state after a few periods of actuation. To compensate for this, x_2 and x_3 were marked on the tube 165.1 mm apart and the time it took for the HDPE ball to go from x_2 and x_3 was manually measured. From this time and measured distances, the net volumetric flow rate was calculated as

$$Q = \frac{0.1651\pi r^2}{t}$$

<div align="right">**Equation 1.**</div>

where r is the radius of the tube and t is the time. The actuation began with the injection phase during which 5 mL of water was injected into the system forcing the same volume of water to enter the Windkessel at different frequencies. Data was collected starting at a frequency 5/6 Hz and increased in increments of 1/6 Hz up to 13/6 Hz. If an air bubble got into system, the tube

would get refilled, and the data was ignored from all prior tests until one set of data could be recorded from 5/6 to 13/6 Hz under the same conditions. Upon completion of one set, the system was refilled to maintain continuity between data sets.

The retraction phase began once the syringe reached 5 mL of water output, causing 5 mL of water being immediately drawn back into the syringe from the Windkessel. The periodicity of this momentum injection and retraction introduces no net-momentum to the system over time despite the observed net-unidirectional flow of the fluid. A diagram of the syringe and actuation over one period is shown in **Figure 2**.
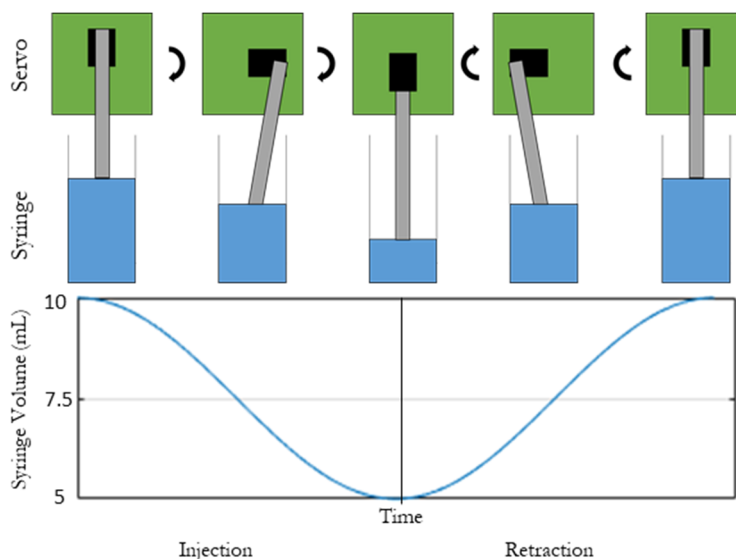
Figure 2. Injection and retraction phases with corresponding syringe and Servo Motor positions.

RESULTS

The experiments showed that a net flow could be generated in a closed system by injecting momentum into the fluid via this zero-net-momentum injection principle. It also showed that the flow rate is highly dependent on the frequency at which momentum is injected. **Figure 3** shows the net volumetric flow rate versus the frequency at which momentum was injected. For each frequency of injection, six data sets were generated.
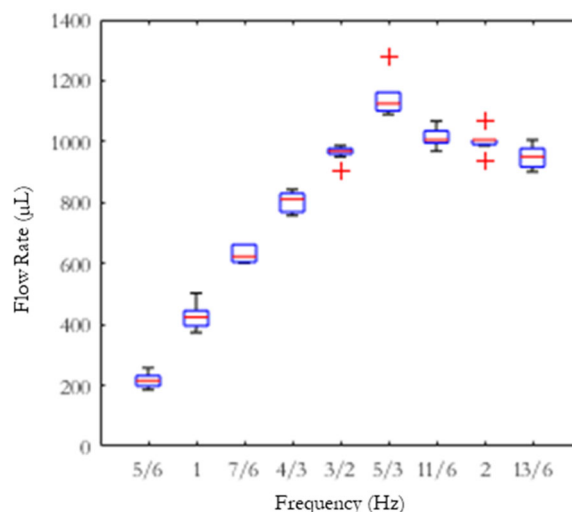
Figure 3. A plot of all of the measured flow rates with respect to the pumping frequency with boxplots of the data for each frequency.

It is important to note that despite the measured net flow, the instantaneous flow rate of the fluid was not constant over time but varied periodically with a mean net flow component. During the injection phase of the pumping, flow was generated in the direction of the net flow. During the retraction phase, however, flow was generated in the opposite direction of the net flow but with a much smaller magnitude. As the frequency increased, the magnitude of the reversed flow during the retraction phase decreased, approaching approximately zero. Swapping the locations of the two syringes as well as changing the Windkessel tilt angle θ to a negative value changed the direction but not the magnitude of the resulting flow.

DISCUSSION
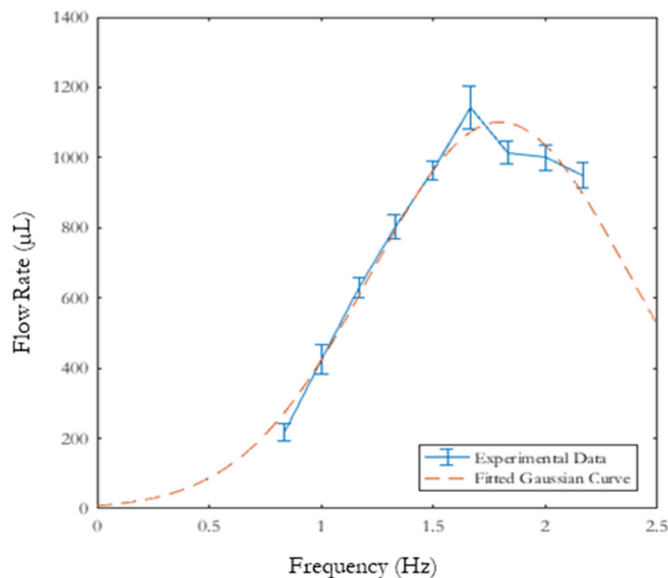
Figure 4. A plot of all of the tests completed with error bars and a fitted Gaussian curve, relating flow rate to the pumping frequency.

The flow rate reached a maximum at 5/3 Hz and then began to decrease showing that there is an optimum actuation frequency that maximizes the flow rate. This result is similar to the theoretical results found in Aboelkassem and Staples[8-12] for a related actuation method where instead of injecting and retracting momentum via fluid, the tube wall collapses and retracts in a zero-net-momentum-injection manner to drive a flow. In those papers, it is established that there is an optimum actuation frequency for that pumping mechanism. A Gaussian curve was fitted to the data and it predicts a maximum flow rate of 1101 µL/s at a frequency of 1.797 Hz. The fitted curve has an *R*-squared value of 0.974 and is plotted with the collected data in **Figure 4**. A Gaussian model was chosen for simplicity since it is everywhere positive and has a single peak like the data. It also serves as a useful experimental comparison to the theoretical work of Aboelkassem and Staples.

To fully understand this pumping mechanism, many more experimental configurations should be completed including but not limited to changing the size of the tube, the fluid being pumped, the tilt angle of the Windkessel and the volumes of fluid in both the syringe and Windkessel. Without this data, stating the scope and scalability of this pumping with full certainty is impossible. However, comparing the presented experimental results to the theoretical work of Aboelkassem and Staples as well as the instances in nature where similar pumping has been demonstrated to exist[6, 8-12], it can reasonably assumed that the pumping for incompressible viscous flow at low Reynolds number (Re $\sim$ δ) can be scaled for channels with a length that is much greater than its width (δ = W/L << 1). For this experiment, the width is the diameter of the tube with the length being the circumference which yields a Reynolds number of 0.00423.

There were two primary sources of error in this experiment. The first is the tilt angle of the Windkessel. While a wedge was used to keep the Windkessel at five degrees during every experiment, the pressures built up inside the system from the higher frequency tests required a lot more force on the Windkessel to hold it in place. This friction caused the hole to deform slightly over time, creating some uncertainty about the exact tilt angle of the Windkessel. This issue could be resolved in future work by using a less deformable material than HDPE for the tubing but an inexpensive, rigid and at least semitransparent material was needed to properly measure fluid flow. Once this zero-net-momentum injection pump is better understood, a stronger and more opaque tube can be used with the same success.

The second source of error was with air bubbles entering the system. These air bubbles would either dramatically reduce the flow or eliminate it entirely depending on the number of bubbles and their size. Although every effort was made to minimize this effect, some smaller air bubbles could not be removed despite the refilling procedure and may have influenced the measured flow rate.

CONCLUSIONS

This paper has investigated a new paradigm in valveless fluid pumping which uses a zero-net-mass injection principle and a periodic mass source and sink to drive a flow in a circular cross section channel. Based on the results presented in **Figure 3**, it is clear that this newly developed method of fluid pumping is successful in creating a net unidirectional flow. Furthermore, by switching the locations of the syringe and Windkessel, the direction of flow could also be changed. Based on the net volumetric flow rate data, a general trend can be observed between the frequency of momentum injection and the volumetric flow rate as seen in **Figure 4**. The flow rate increased as the frequency increased up to a maximum at around 5/6 Hz and then decreased at higher frequencies, which follows the trend found in similar pumping mechanisms[8-12] with a predicted maximum flow rate of 1101 µL/s at a frequency of 1.797 Hz.

One advantage of this pumping mechanism over other valveless pumping mechanisms for future microscale fluid manipulation applications[1-5] is that it does not require the use of sections of hard-to-fabricate elastic tubing, or electric current which can change the chemical properties of the fluid. Additionally, this actuation mechanism can be easily implemented manually, although the results presented were done by a computer-controlled Servo Motor for accuracy. These advantages open up the possibility for pumping fluid cheaply and robustly in a wide variety of applications, particularly in microfluidics where lab-on-a-chip technology is becoming ubiquitous.

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PRESS SUMMARY
Pumping fluids at the microscale have become increasingly important as lab-on-a-chip technology continues to advance. Unfortunately, the existing valveless pumping mechanisms to drive flows at the microscale require internal moving parts or elastic tubing which are both expensive and prone to malfunction. A novel mechanical method to valvelessly pump fluid has been developed via a syringe and a tilted canister that function together to generate a unidirectional flow by periodically injecting and retracting momentum via mass. Experiments involving this pump show that the generated flow rate depends on the frequency of the syringe actuation. This pumping mechanism does not use any internal moving parts or elastic tubing and can even be achieved by simple manual actuation which could have implications in low-cost devices for global health applications.

Sex Bias in Tuberculosis in the Developing World

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ABSTRACT

Tuberculosis (TB), the most deadly global single organism infectious disease, kills nearly twice as many men as women. Understanding the factors that drive this bias in TB mortality is an important aspect of the global effort to reduce the enormous burden of this disease in the developing world. One third of the world's population is estimated to be infected TB, with Low and Middle Income Countries (LMIC) bearing the greatest disease burden. In LMIC sex bias in TB is influenced by sociocultural, behavioural as well as biological factors, with dynamic interactions between reporting variables, other confounding variables and physiological mechanisms, which each influence one another to produce the male-biased sex ratio observed in TB transmission, prevalence and mortality. While confounding factors are addressed in the existing global drive to tackle TB it is the biological aspects of sex bias in TB that present specific challenges for diagnosis and treatment in men and women as they potentially influence future immunological-based interventions to treat TB.

KEYWORDS

Tuberculosis; Low and Middle Income Countries; Sociocultural Influences; Behavioural Bias; Biological Sex Bias; Reporting Bias; TB and Sex Hormones

INTRODUCTION

Global prevalence of tuberculosis

One third of the world's population is estimated to be infected with latent tuberculosis (TB), with 10% progressing to active infection over their lifetime. In 2017, 10 million people fell ill with TB, with 1.3 million deaths.[1] Globally, TB is one of the top 10 causes of death and the leading cause of death from a single infectious agent (above HIV/AIDS). While Africa has the highest incidence rate of TB (236 cases per 100,000 people in 2017) the majority of patients with TB live in the most populous countries of Asia (52%).

TB incidence is correlated with a weak immune system, as this increases the chance of active infection.[2] Factors which increase the chance of active infection are: HIV, malnutrition, smoking and alcohol abuse, and are all associated with living in a LMIC. The common pattern of migration seen in LMIC from rural to highdensity areas, increases transmission rates.[3] Even though a re-emergence of TB in LMIC is related to migration of young adults, the age class with the highest TB rates are adults over 45 years old due, in part, to birth rate decreases as longevity and education improve in LMIC.[4] There has been a resurgence of TB in Eastern Europe since the 1990s with the fall of the Soviet Union and subsequent economic decline and political instability that resulted in an inadequate public health approach to TB control. Limited and substandard quality first and second-line drugs contributed to the increase of multi- and extensively-drug resistant TB (MDR- and XDR-TB) in Eastern Europe. MDR-TB are strains of TB which are resistant to at least two front-line drugs: isoniazid and rifampicin, while XDR-TB is additionally resistant to any fluoroquinolone and at least one injectable second-line drug (i.e. amikacin). Currently, only 3% of the global burden of TB but 20% of the MDR-TB is found in the European region, primarily Eastern Europe.[1]

In Africa, the rapid rise in HIV infection rates in the 1980s contributed to the resurgence of TB on the continent.[5] Normally only 5-10% of infected individuals develop TB in their lifetime, however when co-infected with HIV the risk of reactivating a latent TB infection increases to 5-15% annually.[6] In 2017, 1 in 3 HIV deaths were due to TB and globally 9% of TB cases were co-infected with HIV.[1] The main factor associated with TB recurrence in people living with HIV in Africa was baseline CD4 count.[7] There is significantly lower HIV-specific T-cell function in co-infected individuals compared with HIV mono-infected individuals, which means that the TB bacilli are not restricted to a few infected macrophages. This results in an increased HIV disease progression in TB/HIV co-infected individuals and reactivation of latent TB.

Sex bias in TB epidemiology has been shown to be influenced by many variables including HIV disease progression, as HIV disproportionately impacts young women aged 15-24 years.[8] Social sexual inequality can also influences rates of MDR-TB, as shown in China where its severe sex ratio imbalances at birth[9] have almost certainly influenced its development as a centre for MDR-TB and XDR-TB.[10] Also differences in MDR-TB transmission between the sexes could have an additional impact on these dynamics.

What role does sex bias have on the global tuberculosis epidemic?

Tuberculosis is about twice as prevalent in men as in women (**Figure 1**), with a global male:female sex ratio for TB of 1.7:1 in 2017. Countries with the highest ratios are globally spread, from El Salvador (3.2:1), The Gambia (2.6:1), Moldova (2.5:1) and the Philippines (2.4:1).[1] Sex bias is historically attributed to epidemiological factors, such as cultural and socioeconomic impacts leading to barriers in accessing healthcare.[11] However, the definitive cause of this bias is unknown but biological differences between the sexes are shown to affect susceptibility to mycobacterial infection.[12]
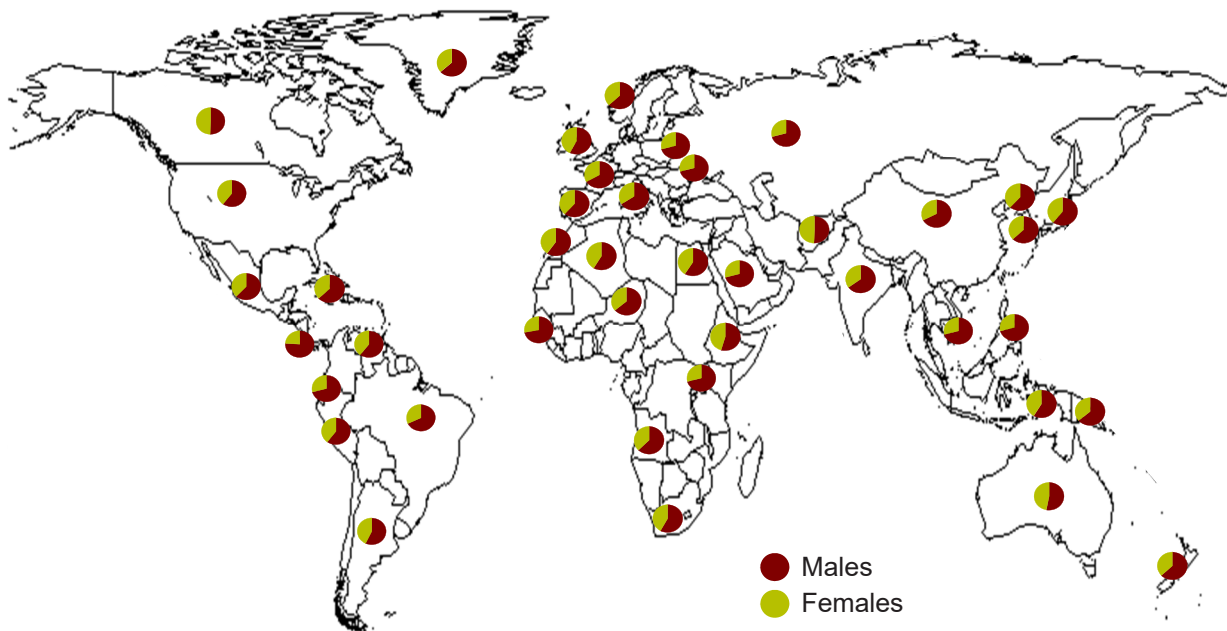
Figure 1. Global sex distribution for new cases of TB in 2017.[1] Males (in dark red) have a greater incidence of TB compared to females (in green) in all regions. El Salvador, The Gambia, Vietnam and some Eastern European countries had some of the highest proportions of TB in males (70-76%).

In an analysis of 29 studies in 14 countries, there were more TB cases in men than women in almost all cases[13] which, as studies of sex bias in TB are predominantly undertaken in LMIC, suggest there is an impact of cultural and social factors. It has been reported that Directly Observed Treatment Short Course (DOTS) for TB, which requires direct supervision of patients taking medication, can result in a greater risk of drug resistance and transmission to contacts among women as some are unable to travel as often as needed to the DOTS clinic.[14] However, a recent global study concluded that sex was not a risk factor for MDR-TB.[15] Given that confounding variables like access to clinics have been shown to affect the viability of treatment[16], the incorporation of progressive treatment of TB into practice, such as working towards women patients administering their own medication, is important.

Studies in High Income Countries (HIC) like the USA can controlled for reporting bias and so enabled trends due to biological differences between the sexes to be examined. One such study by Martinez et al.[17] still showed a higher incidence of TB in men than women (2.1:1) even in HIV-negative populations, with the highest sex ratio in 45-64 year old adults. This is an age group when women are more likely than men to access healthcare[14] so more likely to be identified as TB-positive, hence this study shows real epidemiological differences between the sexes. In addition, Martinez et al.[17] found that the highest sex biases seen were in clusters of patients, meaning that within these clusters there is a higher chance of infection and higher rates of progression from latent to active infection. This conclusion was supported by Cattamanchi et al.[18] who showed that being male was linked with clustering in mycobacterial genetic analyses. These studies conclusively state that differences they detected in TB rates were due to the dynamics of TB transmission and not reporting bias.

Is there evidence that reporting bias affects the sex ratio in tuberculosis?

Healthcare in LMIC is often less accessible to women, due to factors such as childcare and inability to travel alone to appointments[19] leading to the suggestion that, in some cases, the epidemiological differences seen between the sexes could be due to reporting bias rather than biology. There is evidence that existing methods of TB detection have higher failure rates in women.[16] Specifically, the most widely used detection method, the tuberculin skin test, is less sensitive at detecting TB in women than in men[12] and in a randomised trial of TB detection by the sputum smear test found that women completed the test incorrectly significantly more often than men.[20] However, when the instructions for the sputum smear test were clearer, the gap in TB notification rates between the sexes was reduced. In addition, men with a cough were significantly more likely to receive a sputum smear examination than women.[21] Studies in Vietnam have found that female cases were significantly more likely to go undetected, with women being diagnosed on average two weeks later than men due to delays from healthcare providers.[22] These results highlight how, on average, TB notification rates are lower for women than for men even when in contact with healthcare providers.

Conversely, in studies where reporting bias is either eliminated or less likely, there are still clear epidemiological differences between the sexes in prevalence of TB. A study carried out in South India using a report from a rural DOTS programme, found that even though men had higher rates of TB, women were significantly more likely to access healthcare services, be notified under DOTS and comply with full treatment.[23] A national survey in Vietnam found that even though TB cases in women were more likely to be reported than male TB cases, the male to female ratio was still 5.1:1.[24] In these particular studies women had higher access to healthcare, but men still had higher rates of TB cases. In these populations the disparity of prevalence of TB between the sexes must be mostly biological, so is there clear evidence to support a physiological difference between men and women in the prevalence and progression of TB?

Do sex differences only affect disease progression of TB?

Sex bias seen in TB cases may be partly due to differences in disease progression from latent to active TB infection rather than initial transmission of TB. It has been reported that women have higher rates of progression to active infection during their reproductive years, while in men higher rates of progression to active TB occur in their later years of life.[14,25] There are many possible reasons for higher rate of disease progression among young women in their reproductive years. Stresses of pregnancy as well as the natural depression of immune function in order to protect the foetus can contribute to disease progression. However, studies, including case controlled studies, have failed to show a link between pregnancy and TB.[26-28] Newly infected people have a greater chance of progression to active infection than those with an older infection.[29] Higher rates of progression to active TB in reproductive-age women could be linked to the lower rates of TB cases in adolescent women compared to adolescent men. Therefore, women are more likely than men to be newly infected during their reproductive years and so more likely to progress to an active infection. In addition, higher rates of progression to active TB infection among older men could be due to sex specific variables, such as smoking or heavy alcohol consumption, which are more common in men and can lead to reduced immune function and increased disease progression of TB.[30] Thus, evidence shows that women in their reproductive years have higher disease progression than men, so the lower notification rates seen for women appear not to be due to a lower disease progression to active TB.[13]

Can the sex ratio be attributed to confounding variables?

Confounding variables could be the basis of sex bias leading to differential TB rates between men and women. Behaviour has been proven to affect exposure to TB, with men more likely to have a greater number of social contacts and, in LMIC, have professions with higher risks of infection, such as mining, compared to women.[31] Household contact with infected individuals is a strong risk factor for TB[32] but even though women typically spend more time at home in LMIC, men still remain at a higher risk of acquiring TB from a household contact.[33]

The prevalence of HIV/AIDS varies by region and between men and women (UNAIDS, 2014). HIV has been discussed as a variable which impacts differential TB incidence between the sexes in a study carried out in San Francisco. As discussed above, TB rates were higher among men than women even in HIV-negative populations in a study that eliminated reporting bias.[17] HIV/TB co-incidence dynamics can give an insight into sex bias, and can also be influenced by the sex differences seen in HIV/AIDS prevalence. A review on TB sex bias and transmission of HIV in Shanghai found that TB infections of men are linked to higher rates of HIV in the population.[34] From this it is clear that there is a viable case for sex differences potentially affecting TB transmission and the interaction between TB and HIV/AIDS. Fifteen percent of women living with HIV are 15-24 year olds and 80% live in Sub-Saharan Africa. As HIV disproportionately affects young women in Africa, it is critical to investigate whether this variable influences TB disease dynamics in reproductive women. A study in Tanzania found that HIV infection among smear-positive TB cases was higher in women than men in the 15-34 years age range.[35] In Zambia it was found that 74% of women aged 14-24 years were HIV-positive compared to 48% of men of the same age. After 24 years of age, there was a higher number of male TB cases, but the prevalence of HIV was similar in all people with TB.[36] These results show the sex

bias seen in TB in the presence of HIV, and demonstrates that even in cases where HIV is more prevalent in women, TB cases are still higher in men.

Confounding variables, like behaviour, HIV and pregnancy can affect TB disease dynamics. These variables can lead to an increased rate of transmission, as well as increased TB disease progression. The co-related disease dynamics. The implementation of TB testing in HIV clinics could result in increased numbers of successful TB treatments. As much is still unknown regarding the disease dynamics of TB and HIV, and their interactions, diagnostics in low resource clinical settings need to improve rapidly.

Physiological mechanisms of sex bias

While analysis of behaviour offers some explanation for the sex differences seen in TB transmission and prevalence, analysis of physiology provides stark evidence for how biological differences between the sexes result in men being more susceptible than women to TB infection, as well as an explanation for increased disease progression seen in men.

Sex-specific gene architecture

Sex-specific gene architecture has been demonstrated to have a major effect on susceptibility to TB infection and disease progression in humans[37], while the use of rodent models has enabled investigation of specific genes known to respond to mycobacterial infection.[38] Genome-wide linkage analyses have identified dominant loci on chromosome 8 and the X-chromosome that appear to confer a predisposition to TB in adults.[39,40] Polymorphisms of the transport protein NRAMP1 were linked to reduced TB susceptibility in young adult women[41] and that the risk of developing TB was linked to polymorphisms in specific toll-like receptors (TLR), which are responsible for cellular recognition of microbial structures and so influence immune response to infection.[42] X chromosome-linked TLR8 gene polymorphisms have been linked to susceptibility to TB infection especially in male children[43] and 2 other X-chromosome linked genes, *IKBKB* and *CYBB,* are part of a group of 9 genes known to be associated with susceptibility to mycobacterial disease.[44] Gene-dosing effects in women have been proven to be beneficial in terms of reduced TB susceptibility as about 15% of X-linked genes can escape silencing. This can lead to the increased expression of these genes in women[45], including immunomodulatory micro-RNAs (miRNAs), which are far more numerous on the X chromosome compared to the Y chromosome.[46] It is clear that investigation of expression of X-linked genes and miRNAs could lead to a better understanding of sex-specific susceptibility to TB infection.

The role of Sex steroids and nutrition in differential TB susceptibility.

In many infectious diseases females exert a greater immune response to foreign antigens than males[44], with sex steroid hormones playing a role in this differential immune responses (**Figure 2**[11,47]). Therefore, sex hormones could be a significant factor for sex bias in TB. Testosterone impairs pro-inflammatory cytokine production[48], whereas oestrogens are a pro-inflammatory inducer.[49] In TB-infected mouse models, non-castrated males had higher mortality, higher bacilli burdens, less inflammation in lung compartments and lower cytokine production compared to female and castrated male mice.[50]

Activated macrophages are thought to have an important role in the immune response to TB infection through the destruction of *M. tuberculosis* (Mtb) bacilli.[51] While the oestrogen sex steroid estradiol enhances macrophage activation[52], testosterone reduces macrophage activation through down-regulation of TLR4 expression.[44] Apoptotic cell death of Mtb-infected macrophages is vital for Mtb control and decreases bacterial growth, whereas necrotic cell death promotes bacterial growth. Apoptotic and necrotic cell death is balanced through the regulation of prostaglandin (PGE2; pro-apoptotic) and lipoxin A4 (LXA4; pro-necrotic) activity.[53] The sex hormone progesterone can increase PGE2 production by monocytes, while testosterone inhibits PGE2 production[54] suggesting sex differences in macrophage regulation and a basis for sex differences in TB infection and progression.

While macrophages appear to have a central role in TB immune response, neutrophils are thought to be the dominant infected cell type in the upper respiratory tract during active TB.[56] Lyadova[57] suggested that TB disease dramatically alters neutrophil population, leading to the accumulation of heterogeneous subsets of immature and activated dysfunctional cells and a decline in true neutrophils. Interferon (IFN) secretion is shown to be driven by neutrophils in active TB infection.[58] Mice lacking IFNγ or the IFNγ receptor showed increased susceptibility to Mtb infection through increased recruitment of Mtb-infected neutrophils into the lungs,[59] highlighting the importance of IFNγ in TB. Neutrophil recruitment can be modulated by miRNA, particularly the X-chromosome linked miRNA-223 which downregulates CXCL2 and CCL3 in neutrophils, and so reduces their recruitment into lungs. Deletion of miRNA-223 results in increased susceptibility to Mtb infection due to pathogenic neutrophil recruitment in the lungs, leading to tissue damage.[60] Incomplete gene silencing in females often leads to higher expression levels of miRNA-223 and so lower pathogenic recruitment of neutrophils compared to males. Progesterone and oestrogen decrease spontaneous neutrophil apoptosis in females,[61] whereas testosterone increases neutrophil activation in males.[62] Sex steroids have also been linked to structural and histological differences of the upper airway and respiratory tract between the sexes.[63,64] Therefore, understanding the influence of sex steroids on immune responses and physiology are critical to determining the biological basis of sex bias in TB.
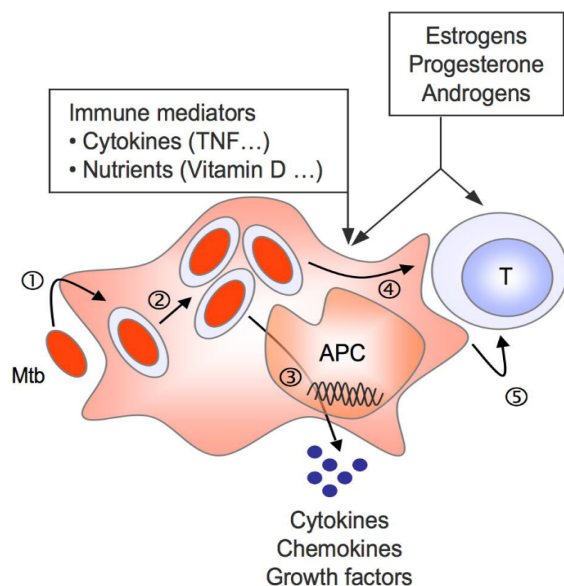
Figure 2. How sex steroids influence mycobacterial immunity. Immune cells and T-cells express receptors for steroid hormones on their cell surface.55 Sex steroids can influence *M. tuberculosis* (Mtb) entry (1) and intracellular trafficking (2) in host phagocytes and antigen-presenting cells. Sex steroids can modulate the secretion of cytokine and growth factors by Mtb infected cells (3), can result in antigen presentation (4) and T-cell development (5). Figure taken from Neyrolles & Quintana-Murci (2009).[11]

Nutrition and metabolism could impact the susceptibility to Mtb infection, as sex differences in nutrition can be linked to immune function.[65,66] In the developing world malnutrition is a major concern, for example, iron deficiency in women is an important issue in LMIC.[67] Studies have shown that iron overload increases susceptibility to Mtb infection, both *in vivo*[68] and *in vitro*.[69] Iron is a critical component of microbial enzymes and redox systems[70] and has been linked to immune system mechanisms to control pathogens, such as mycobacteria.[71] Vitamin D has been reported to mediate an antimycobacterial response through the triggering of TLRs[72] and oestrogen has been linked to vitamin D-mediated resistance.[73] A case-control study among Gujarati Asians living in west London, UK, found that vitamin D deficiency and receptor polymorphisms had a major influence on susceptibility to TB.[74]

Sex differences in treatment
As physiological differences between the sexes can result in differential susceptibility to Mtb infection and progression to active infection, it is possible that treatment would have different outcomes between the sexes due to sex-specific mechanisms as well. For example, a Ugandan study investigating sex differences in the presentation and outcomes of HIV-infected adults with TB, found that there were differences of TB incidence rates between the sexes at presentation. However, 1 year after the start of TB treatment outcomes were similar, suggesting there were no differential treatment effects between men and women.[75] In contrast, a study in West Bengal, India, found there was a statistically significant difference between treatment outcomes in men and women, with more women successfully treated compared to men.[76] In both of these studies, confounding factors were eliminated indicating it is still unclear whether sex-bias influences success rates in TB treatment.

Social factors can also influence treatment outcome, as completion of TB treatment has been reported to be affected by various factors including sex and impact of HIV/AIDS[77], although these data are somewhat contradictory. Hudelson[14] reported lower treatment compliance of women compared to men as women were impacted more severely by the stigma associated with TB. In contrast, a four-country (Bangladesh, India, Malawi and Colombia) WHO study found that there were higher dropout rates in men than women due to distress caused by fear of stigma and rejection.[78] Financial impact, hospitalization due to the disease and difficulties in attending clinics during opening times are suspected to be major causes for failure in TB treatment completion in men[23] and higher dropout rates among males than females were also reported in a more recent study in Taiwan.[79]

A systemic review of qualitative research highlighted major social and cultural factors which influence TB treatment outcome, including poverty, gender discrimination and health service factors.[77] This review found a correlation between the patients' understanding of the treatment and a positive treatment outcome. In this review women were more motivated to adhere to treatment, which aligns with reports from other studies on health-seeking behaviour. Failure to complete first-line treatment is linked to the development of drug resistant Mtb strains. Previous treatment with a second-line injectable drug is the strongest risk factor for XDR-TB[80], however various studies have linked being female as another major risk factor for MDR and XDR-TB.[81,82]

Treatment needs to be offered in ways that maximises patient adherence to improve positive TB treatment outcome rates. Social factors due to work schedules of men and fear of stigma for women need to be considered and addressed, in order to provide appropriate interventions for both sexes and reduce treatment dropout rates. Physiological mechanisms of sex bias need to be better understood so that healthcare providers can offer optimal treatment for each sex to improve cure rates.

CONCLUSION

Despite recent efforts TB is still a leading cause of death worldwide, killing more than a million individuals each year. Though seemingly dormant in developed countries since the introduction of the BCG vaccine in the 1950s, TB re-emerged in parts of Europe and the developing world due to various confounding variables. The rise of HIV in Africa in the 1980s led to a rapid rise in TB rates, while socio-economic decline from the fall of the Soviet Union in the 1990s resulted in poor living conditions and healthcare provision, leading to MDR/XDR-TB. With increasing global migration and mobility between regions a rise in TB notification rates in the developed world is likely to occur in the future.

The 2018 WHO Global TB report, documented a staggering 54 million lives saved between 2000 and 2017 due to effective diagnosis and treatment, alongside a 42% drop in mortality due to TB.[1] The evidence discussed here points to an epidemiological sex bias in the transmission and prevalence of tuberculosis. A complex mixture of biological and cultural factors underlie a stark differential susceptibility to TB infection and progression to disease between males and females. Reporting bias due to cultural factors can contribute to lower notification rates of TB infection in women[19], however reporting bias is not strong enough to explain the differences in male and female susceptible to TB. HIV is known to modulate TB susceptibility[5], so high rates of HIV in regions such as Sub-Saharan Africa[83] are likely to influence TB rates in these populations. However, higher TB rates in men than women in HIV-negative populations[17] show that sex bias is important in TB epidemiology.

Much work needs to be done to understand the relationship between sex and TB, as the physiological mechanisms behind immune responses and immunity to TB in men and women are unclear. It is highly likely that there is an interplay between physiological mechanisms influenced by sex-specific gene architecture, differential immune responses, and nutrition alongside confounding variables influenced by cultural factors, HIV prevalence, and MDR/XDR-TB prevalence, which are responsible for the male-biased sex ratio reported globally in TB. Improved knowledge of the role of sex steroid hormones in immunity will greatly improve understanding of how sex influences resistance to TB.

The WHO End TB Strategy plans to end the global TB epidemic, with targets to reduce TB deaths by 95% and to cut new cases by 90% by 2035.[1,84] Reaching these targets could be assisted by immunological and epidemiological research of sex bias. These future developments could impact the intervention of vaccines and other immunological approaches to improve TB cure rates, and ultimately lead to better education and knowledge of TB transmission and disease progression between the sexes, resulting in improved TB notification rates in men and women and a more effective strategy to tackle TB globally.

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PRESS SUMMARY
One third of the world's population is estimated to be infected with tuberculosis (TB), a disease that affects twice as many men than women. We consider the different sociocultural, behavioural and biological factors that may produce this bias and consider how this might affect TB Transmission and progression and the possibility of exploiting these sex differences to better target TB treatments.

Obstructive Sleep Apnea is Associated with Longitudinal Increases in Amyloid Burden in Elderly Mild Cognitive Impairment Individuals

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ABSTRACT

Cross sectional analysis has shown an association between Obstructive Sleep Apnea (OSA) severity and Aβ burden using amyloid-PET among Mild Cognitive Impairment (MCI) patients. However, whether OSA accelerates longitudinal increases in amyloid beta (Aβ) burden in MCI patients is presently unclear. Study participants included a total of 798 subjects with a diagnosis of MCI and were a subset of the ADNI cohort (adni.loni.usc.edu). OSA was self-reported and participants were labeled either as OSA+ or OSA−. Aβ burden was determined by florbetapir SUVRs. To test whether OSA is associated with the rate of change in Aβ data longitudinally, multilevel mixed effects linear regression was used to fit the models with randomly varying intercepts and slopes allowing dependence on OSA status. The final model was adjusted for age, sex, body mass index, education, CPAP use status, history of respiratory disease, hypertension, diabetes, and history of cardiovascular disease. A significant variation in the change (slope) in Aβ volumes over time was seen (p<.0001). The covariance between the baseline Aβ level and Aβ volume change over time indicated that OSA subjects experienced greater mean change differences in brain Aβ volumes over time (p < .0001). The rate of change in Aβ deposition also varied significantly across OSA groups over the follow-up period. Obstructive Sleep Apnea possibly facilitates longitudinal increases in amyloid burden in elderly Mild Cognitive Impairment individuals. Further research examining mechanisms underlying effects of OSA on the longitudinal increases in Aβ burden is needed.

KEYWORDS

Obstructive Sleep Apnea; OSA; Amyloid; Mild Cognitive Impairment; MCI; Elderly

INTRODUCTION

Alzheimer's Disease (AD) and Obstructive Sleep Apnea (OSA) are both highly burdensome chronic diseases. OSA affects a predicted 23.4 % of middle-aged women and 49.7% of middle-aged men, though estimates vary. The disease is associated with significant morbidity, and frequently goes undiagnosed.[1] AD affects an estimated 5.4 million Americans and costs $236 billion per year to provide care.[2] Development of preventative measures is therefore imperative to reduce this societal burden.
Mild Cognitive Impairment (MCI) is a condition in which memory loss is beyond the scope of normal aging, but is not severe enough to meet the criteria for AD.[3] MCI is frequently a precursor to AD and is therefore an ideal stage for preventative intervention.[3,4] Factors that alter the progression from MCI to AD can be studied using biomarkers such as amyloid beta (Aβ).[5] Aβ begins accumulating long before the onset of symptomatic AD and has been supported as a reliable marker of disease progression.[6]

OSA is characterized by intermittent obstruction of the upper airway during sleep resulting in hypoxia and sleep fragmentation. It is treatable using continuous positive airway pressure (CPAP). Past research has indicated a correlation between untreated OSA and cognitive decline.[7,8] Studies have further associated worse sleep quality with increased Aβ deposition in humans.[9,10] Moreover, one study found the presence of OSA to be associated with the development of cognitive impairment at follow up approximately five years later.[11] Furthermore, a recent study demonstrated that objectively measured OSA was associated with markers of increased amyloid burden over a 2-year follow-up in the NYU cohort that consisted exclusively of community-dwelling healthy cognitively normal elderly.[12] However, whether OSA accelerates longitudinal increases in Aβ burden in MCI patients is presently unclear.

Therefore, we hypothesized that OSA would facilitate longitudinal increases in brain amyloid beta deposition in individuals with MCI. Such a finding would consolidate understanding of the relationship between OSA and cognitive decline as well as further focus prevention efforts.

METHODS

ADNI Dataset

We downloaded data from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (*http://adni.loni.usc.edu/*) on December 7, 2016. ADNI is a longitudinal multisite study, which seeks to confirm that MRI, PET, and other biomarker data in conjunction with clinical and neuropsychological assessment can be used measure the progression of MCI and AD. Subject data was collected through approximately 50 universities and medical centers in the United States and Canada. Follow up was performed at 6-month intervals for approximately 3 years. Each site received IRB and radiation safety committee (RSC) or radioactive approval, before scanning subjects. A written informed consent was obtained from all participants.

Participants

We used the subset of the cohort with mild cognitive impairment for analysis. Seven hundred and ninety-eight (798) individuals were included. The criteria for MCI classification were as follows: (i) Mini-Mental State Evaluation (MMSE) score in the range of 24-30; (ii) CDR score of 0.5 with a minimum of 0.5 on the memory box score; (iii) On one paragraph from the Logical Memory II subscale of the Wechsler Memory Scale- Revised (maximum score of 25), a minimum score of 8 for 16 years of education, 4 for 8-15 years of education, and 2 for 0-7 years of education. Additionally, MCI individuals must have had memory complaints, while largely maintaining general cognition and functional performance. They could not qualify for diagnosis of dementia.

Subjects were excluded if: (i) "Insomnia" or other unspecified sleep disturbances were present, because lack of sleep has been independently associated with cognitive decline;[13] (ii) Past surgery to treat OSA was reported; (iii) A change in body mass index (BMI) greater than 5 between visits was observed, because BMI is independently associated with both OSA and cognitive decline;[14] (iv) MCI diagnosis was reversible; or (v) Data was missing for any important covariates such as APOE4 status, history of cardiovascular disease, or CPAP status.

OSA Diagnosis

A clinical interview was conducted with each participant in order to determine OSA status. If a participant reported a previous clinical diagnosis of "sleep apnea", "sleep disordered breathing", "OSA", or "SDB", then they were classified as OSA+. The remaining participants were classified as OSA-. To ensure correct classification, three physicians (OQU. FM. And OM.B.) reviewed the medical history clinical notes and confirmed group placement.

Characteristics	All	OSA-	OSA+
Number of participants (%)	798 (100)	695 (87)	103 (13)
Female gender, number (%)	319 (40)	297 (43)	25 (25)
Age, years, median (interquartile range)	74 (68, 79)	71 (70, 78)	71 (70, 76)
APOE positive, number (%)	410 (51)	368 (53)	42 (41)
Education, years, median (interquartile range)	16 (14, 18)	16 (14, 18)	16 (14, 18)
BMI (kg/m2)	26.9 ± 4.6	26.5 ± 4.4	29.0 ± 5.3
Hypertension, number (%)	395 (49)	337 (48)	58 (56)
Diabetes, number (%)	75 (9)	57 (8)	18 (17)
Thyroid Disease, number (%)	156 (20)	133 (19)	23 (22)
Respiratory Disease, number (%)	195 (24)	133 (19)	62 (60)
Aβ, median (interquartile range)	1.2 (1.0, 1.4)	1.2 (1.0, 1.4)	1.1 (1.0, 1.3)

Table 1: Descriptive characteristics of MCI Participants by Obstructive Sleep Apnea status.
Abbreviations: Aβ: Amyloid β; APOE: Apolipoprotein E; BMI: Body Mass Index

Amyloid PET

Florbetapir (18F-AV-45) PET data was obtained and processed as previously described elsewhere.[15,16] Briefly, images were acquired in 5-minute scans repeated four times, 50-70 minutes after florbetapir injection. Resulting images were realigned, averaged, interpolated to a common voxel size (1.5 mm3), and smoothed to a common resolution (8 mm3 in full width at half maximum). Mean florbetapir standard uptake value ratios (SUVRs) were created by averaging the lateral and medial anterior frontal, posterior cingulate, lateral parietal and lateral temporal regions and then normalized to a cerebellar reference region.

Statistical Analysis

Analyses were performed using SAS 9.3.[17] All variables were plotted for assessment of outliers. Frequency distributions of all variables were also assessed. Descriptive statistics were calculated for demographic and clinical data at baseline. Characteristics of the study groups by OSA status was compared using Pearson's Chai Squared test and for all categorical variables e.g. gender. T

test was used for continuous variables. Bivariate analysis was conducted to describe the distribution of OSA status across categories of all potential confounders and clinical covariates e.g. age, sex. Potential confounders were identified and included in the two stage multi-level mixed effect linear regression model (described below) if exclusion or addition of a covariate to the full model caused a change in the adjusted Aβ estimates of OSA status by 10% or more.

Multivariate ANOVA was used to assess for differences in time trend groups (OSA+ *vs*. OSA-) and time points. The ADNI data was unbalanced with unequal numbers of measurements for each participant. Therefore, in order to minimize bias, multilevel mixed effects linear regression models with normal errors were used to analyze the rate of change of Aβ volume longitudinally based on OSA status.[18-20] PROC MIXED was used to fit the model with randomly varying intercept and slopes, allowing dependence on OSA status. The final model was adjusted for age, sex, body mass index, education, CPAP use, APOE e4 status, history of respiratory disease, hypertension, diabetes, history of traumatic brain injury, and history of cardiovascular disease (including ischemic heart disease, heart failure, and stroke/TIA).

Sensitivity analysis removing self-reported CPAP-users (n=16) from OSA+ participants had a negligible impact on the estimates (e.g. Aβ burden estimate of .06 changed to .08). Power issues as well as insufficient follow-up data points hindered conducting the same complex analyses comparing longitudinal changes in biomarkers between OSA patients treated by CPAP and OSA patients not under CPAP treatment.

RESULTS

At baseline, the median (interquartile range) age of participants was 74 (68, 79). Females accounted for 319 (40%) of the participants and the median (interquartile range) years of education was 16 (14, 18). The average BMI was 26.5 ± 4.4 for those without OSA and 29.4 ± 5.3 for those with OSA (Table 1).
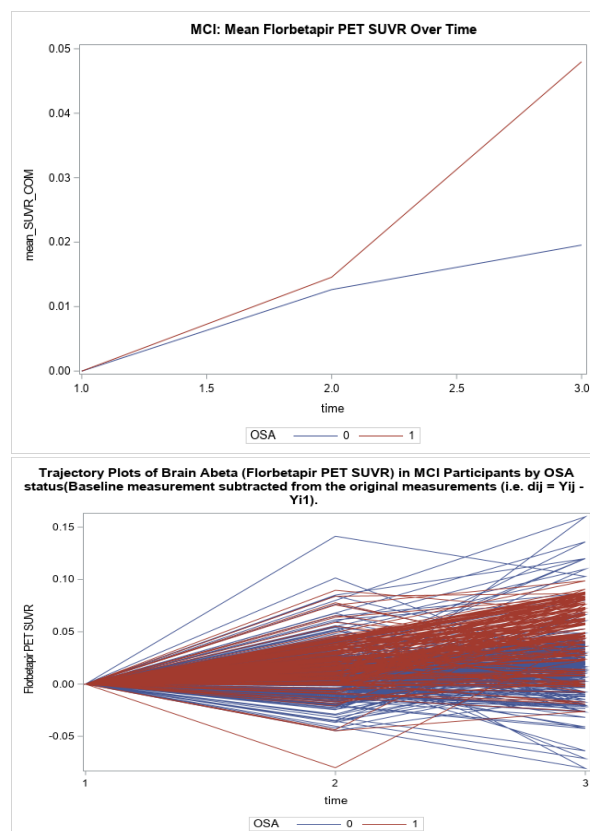
Figure 1. Multivariate Analysis of Variance showing Brain Aβ-42 Differences in Time Trend, Groups (OSA+ versus OSA-) And Time-points by OSA status 0=OSA- and 1=OSA+.
Y-axis: MCI: Mild Cognitive Impairment; SUVR: Standard Uptake Value Ratio of Brain Florbetapir; mean_SUVR_COM: Mean change Brain Florbetapir Uptake Value; Florbetapir PET SUVR: Florbetapir Positron Emission Tomography Standard Uptake Value Ratio of Brain Florbetapir
Y-axis: pg/ml; X-axis: Time 1= baseline, Time 2=Year 2 value minus baseline, and Time 3= Year 3 value minus baseline.

Figure 1a-b shows results from MANOVA showing Brain Aβ-42 differences in time trend, groups (OSA+ *versus* OSA-) and time-points by OSA status. The MANOVA time trend of the Aβ levels showed that the change trend was not parallel across groups as shown by the group*time interaction term (Pillai's Trace test p value=.0143). Across all subjects, the mean Aβ levels increased significantly over time. Significant differences in mean brain Aβ across OSA status existed when each time point was compared to the previous time point (time point 1 p value<.0001; time point 2 p value=.0137).

Manova Test Criteria	Statistic	Value	F-value	P-value
Timepoint* OSA Effect	Pillai's Trace	0.047	4.24	0.0143*
Timepoint Effect	Pillai's Trace	0.129	18.06	<.0001***
Repeated Measures Analysis of Variance of Contrast Variables OSA+ v OSA-				
Timepoint_1 (Year 2)			0.08	0.7786
Timepoint_2 (Year 3)			4.63	0.0323*
Timepoint_1 v timepoint_0			25.63	<.0001***
Timepoint_2 v timepoint_1			6.16	0.0137*

Table 2: Multivariate ANOVA Results Testing Biomarker Mean Change Differences in Time Trend, Groups and Time Points.
*P-value <=.05
**P-value <=.01
***P-value<=.001

Multilevel mixed effects regression modeling showed significant variation in the change (slope) in Aβ-42 volumes over time (B=.08, 95% CI=.05, .12, p-value<.0001). The covariance between the baseline Aβ-42 level and Aβ-42 volume change over time indicated that OSA subjects experienced a faster increase in brain Aβ-42 volumes over time (B=-.06, 95% CI= -.09, -.04, p-value<.0001). The rate of change in Aβ-42 deposition also varied significantly across OSA groups over the follow-up period.

Parameters	Estimate	95% CI	P-value
OSA+ *vs.* OSA-			
β- Amyloid Burden at Baseline (intercept)	0.06	.03, .09	<.0001
β- Amyloid Burden over time (slope)	0.08	.05, .12	<.0001
β- Amyloid Burden over time (covariance)	-0.06	-.09, -.04	<.0001

Table 3: Between Subject Variation in β-Amyloid Deposition in Mild Cognitive Impairment.

DISCUSSION

This longitudinal study found that individuals with OSA experienced an increased rate of Aβ deposition over time (average follow-up time was 2.52 ± 0.52 years), compared to non-OSA participants.

These findings are consistent with past studies, which have indicated a cross- sectional association between OSA and Aβ and an increased incidence of AD in OSA individuals. Cross- sectionally, Spira, *et al.* found that sleep deprivation was correlated with increased brain Aβ levels in community dwelling older adults.[10] Both Bu et al and Ligouri et al found that untreated OSA was associated with AD biomarkers.[21,22] OSA was further found to be associated with earlier onset of AD by Osorio, *et al.*[23] In a recent meta-analysis, Bubu et al found that those with OSA were at a 2.37 times higher risk for cognitive impairment or AD.[7] Based on such studies it has been hypothesized that OSA may facilitate greater rate of Aβ deposition over time. To our knowledge, our study is the first to examine Aβ deposition longitudinally in OSA+ and OSA- individuals in an MCI cohort to support this hypothesis.

Past research has proposed mechanisms that may explain the increased rate of amyloid deposition that was observed. Hypoxia, a hallmark of OSA, has been shown to contribute to increases in Aβ production.[24] In mice, hypoxia treatment was correlated with an increased levels of brain Aβ, which indicates a potential relationship between hypoxia and AD pathogenesis.[25] Disruption of the sleep cycle, another characteristic of OSA, may also contribute to decreased Aβ clearance and therefore greater accumulation.[26] We did not specifically examine MRI measures of brain atrophy in this study, however, our findings suggest that in OSA patients, hypoxia or sleep fragmentation significantly affects brain Aβ changes, which parallels neurodegeneration that in turn may drive the rate of cognitive decline.

Strengths and Limitations
This study possesses several strengths including a well-defined cohort, and objective assessment of β-amyloid burden, which allowed for a high degree of certainty regarding measurement of our outcome. In addition, our statistical analytic methods were robust with respect to unbalanced number of observations per subject over time.

A limitation of this study is the self-reported nature of OSA. Self-reported sleep measures can be influenced by cognitive deficits and in certain situations might not be correlated with objective sleep measurements.[27,28] Furthermore, reported OSA prevalence in our cohort was lower than the OSA prevalence in the elderly population suggested by epidemiological and sleep laboratory

studies. This can relate to an underdiagnosis effect and most likely indicates that some OSA+ individuals were incorrectly categorized as OSA-. Most likely, such an error would have driven our findings towards the null. However, one implication of OSA classification by self-report is that those who sought diagnosis most likely did so because they were experiencing associated symptoms. The prevalence of OSA in our cohort (6%) is similar to the U.S. prevalence of OSA Syndrome (4%), which is defined by both AHI ≥5and daytime symptoms (i.e. excessive daytime sleepiness).[29] The prevalence of OSA with or without symptoms in the elderly is much higher (estimated at 30-50% in older subjects.[30] This is significant because the associated symptoms of OSA may also impact Aβ accumulation. Notably, all-cause excessive daytime sleepiness in elderly subjects defined by Epworth sleepiness scores ≥10 was associated with longitudinal brain beta amyloid accumulation in a recent study.[31] Therefore, additional work may be required to differentiate the risk of OSA for AD with and without associated daytime symptoms

Our results have potentially significant public health implications. Since the majority of OSA goes undiagnosed, our findings further highlight the need for increased screening and treatment of obstructive sleep apnea. Individuals with mild cognitive impairment are an especially high priority for intervention, as OSA treatment could potentially slow their cognitive decline. Controlled intervention trials are needed to confirm the efficacy of OSA treatment (*i.e.* CPAP) in reversing the increased rate of Aβ deposition.

CONCLUSIONS

A faster rate of amyloid beta deposition was seen in individuals with obstructive sleep apnea compared to non-OSA participants. Further studies are needed to replicate this finding using more objective sleep measures. In MCI elderly, clinical interventions aimed to treat OSA are needed to test if OSA treatment can affect the progression of cognitive impairment due to AD.

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PRESS SUMMARY

Obstructive sleep apnea (OSA) is a highly prevalent, treatable, and frequently undiagnosed disease in the United States today. OSA has previously been cross-sectionally associated with a hallmark of Alzheimer's Disease—amyloid beta burden in the brain. Whether this relationship can be seen longitudinally has remained unclear. In this study, the amyloid beta levels of 798 subjects were tracked over about three years as a part of the Alzheimer's Disease Neuroimaging Initiative. Amyloid beta accumulated at a faster rate in subjects with OSA as compared to controls. This indicates that OSA could be facilitating cognitive decline. If so, then OSA is a possible target for therapeutic intervention for slowing progression to Alzheimer's Disease. Further research replicating these findings using objective sleep measures and investigating mechanisms is needed to support these conclusions.

It Gets Better with Time: The Perception of Stigma Among Older Adults with Chronic Physical Illness and in Recovery from Mental Health Condition

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ABSTRACT

Older adults are a vulnerable population who are more susceptible to developing mental health conditions, and the symptoms are often exacerbated by the co-occurrence of various physical health complications. Despite available evidence-based interventions, many older adults neglect to utilize mental health services, due to the stigmatization of mental health conditions. Limited research has focused on the unique experiences of older adults who have overcome the adverse effects of stigma, have sought help for their mental health condition and are currently in recovery. There are even fewer studies that have addressed perceptions of stigma among older adults in recovery from a mental health condition who are currently living with a chronic physical illness. The present study investigated the unique experiences of seeking professional mental health services and the perceptions of stigma among nine older adults living with a chronic physical illness and currently in recovery from a mental health condition utilizing semi-structured interviews. Through an in-depth thematic analysis of the data, four over-arching themes were identified: Resilience from the Stigma of a Mental Illness, Community Engagement, Cultural Barriers and Social Support System. Findings from the current study suggest that older adults who have previously experienced a mental health condition and were able to overcome the stigma of their condition, were more likely to seek professional help. Additionally, engaging in community engagement programs to help other older adults who are currently experiencing acute mental health conditions seemed to reduce perceptions of stigma and positively impacted participants self-esteem and overall outlook on life.

KEYWORDS

Stigma; Perception; Older Adults; Mental Health Condition; Co-Occurring Disorders

INTRODUCTION

Older adults are the fastest growing segment of the US population, and by the year 2040, the number of adults 65 and older is projected to be 80 million.[1] Older adults are susceptible to the development of various mental health conditions. Researchers' have projected approximately 15 million older adults will have a diagnosable mental health condition by the year 2030.[2,3] However, due to the high percentage of mental health conditions going under-diagnosed in the older adult population, these future prevalence estimates are likely an under-estimate of what we can expect.[1]

Depression is the most prevalent mental health condition among older adults, affecting one in ten individuals.[4-7] Depression is characterized by intense feelings of sadness, disturbed sleep or appetite, fatigue and poor concentration.[8] According to the Diagnostic and Statistical Manual of Mental Disorders, depression is diagnosed when the changes in one's personal, vocational, social and health functions are clinically significant, lasting for a minimal duration of two weeks[9]. Although the onset of depression can develop during any stage in life, depression often goes undiagnosed and undertreated well into later adulthood[6]. Late-life depression is associated with longer hospital stays, functional limitations, higher mortality rates, and higher medical co-morbidities in comparison to other medical disorders.[6,10] Other common mental health conditions in this population include: schizophrenia, delusional disorders, bi-polar disorder, anxiety and depression.[4,6] The debilitating effects of mental health conditions include: substantial loss in the quality of life in older adults, financial burden and the disproportionately higher mortality rates of those with a mental health condition compared to those without mental health conditions.[11,12]

Another issue faced by older adults living with a mental health condition is the exacerbation of mental health symptoms due to the co-occurrence of a chronic physical illness. The co-occurrence of physical and mental health conditions is frequent in older adults; approximately 80% of older adults have at least one chronic physical health condition, and 50% have two or more chronic

physical health conditions, which are often accompanied with years of pain and loss of function.[4] Individuals with a mental health condition have an increased risk for the development of chronic physical medical conditions, compared to those without a mental health condition.[13] Individuals with co-occurring physical and mental health conditions face more prejudicial attitudes from members in their communities, and are more likely to avoid utilizing mental health services compared to individuals with solely a mental health condition.[14] Clinicians are often guided by the negative stereotypes of mental health conditions, and are more likely to treat physical health complications of their patients with a mental health condition less thoroughly and effectively.[15] Stigma continues to be a world-wide health concern and older adults living with a mental health condition continue to be victimized by the effects of stigma.[14] Stigma regarding a mental health condition can be divided into three components: public, perceived and internalized-stigma. Public stigma refers to the prejudicial attitudes of members in communities toward individuals with a mental health condition.[16] Perceived-stigma occurs when stigmatized individuals believe that others hold negative stereotypes toward individuals with a mental health condition.[17] Internalized stigma occurs when stigmatized individuals believe the stereotypes about individuals with a mental health condition are true, and apply those beliefs to how they feel about themselves.[18]

Stigma associated with a mental health condition is associated with mistreatment, rejection, social distancing, fear and ill treatment from members in society.[19, 20] Individuals who may benefit from mental health treatment choose not to pursue services or begin treatment but drop-out prematurely to avoid prejudices and discrimination associated with having a mental health condition. [21-24] Help-seeking behavior is often seen as a sign of personal weakness, contributing to the reason why many individuals seek professional help as a last resort.[20] Although research suggests stigma associated with the utilization of mental health services is a greater barrier than the condition itself, there are individuals who have found ways to overcome those barriers.[20]

Current literature on the stigmatization of a mental health condition frequently highlights the perception of stigma among individuals currently experiencing a mental health condition, but such research often omits the population of older adults in recovery from a mental health condition. There are even fewer studies that have addressed perceptions of stigma among older adults in recovery from a mental health condition who are currently living with a chronic physical illness. The present study begins to address this gap in the literature by providing a deeper understanding of the perception of stigma among older adults who self-identified as being in recovery from a mental health condition. This research is central toward helping us understand the mechanisms through which some vulnerable populations of elders have been able to break through the barriers of stigma and engage in professional mental health services. Information from this investigation can assist in identification of targets toward helping other vulnerable populations of older adults combat the effects of stigma.

METHODS
The present study investigated the unique experiences of seeking professional mental health services and the perceptions of stigma among older adults living with a chronic physical illness and in recovery from a mental health condition. Qualitative research methodology was used to gain an in-depth understanding and to explore the unique experiences of the study participants. Qualitative data has been widely used in the field of psychology to assess various mental health related topics[25]. Semi-structured interviews were administered to allow participants to give a broad and detailed description of their experiences of stigma. The use of semi-structured interviews allows for the emergence of themes that may not be predicted ahead of time by the researcher due to the participant's opportunity to speak freely in a comfortable environment[26].

Setting and participants
Study participants were recruited from the *"Enhancing the Care Transitions Intervention to Facilitate Wellness and Reduce Hospital Readmissions among Older Adults with a Co-occurring Mental Health Diagnosis"* pilot study (PI: Kyaien Conner). All participants (N=9) in this study responded to flyers located at the local Area Agency on Aging (AAA) asking for older adults who were in recovery from a mental health condition and were interested in being trained to work with other older adults currently experiencing acute mental health conditions. Inclusion criteria included older adults who: (a) were 55 years of age or older; (b) self-identified as having a previous mental health condition and currently being in recovery; and (c) self-identified as having a current chronic physical illness. All participants were informed about the voluntary nature of the study and received a written informed consent document to read prior to study engagement. Participants were told that their participation in this study would in no way affect their participation in the larger ENHANCE study. All activities complied with the University of South Florida's provisions for the ethical conduct of research with human subjects (CR1_Pro00025016).

Demographical characteristics
Participants self-reported items regarding their identity including age, race, ethnicity, education, gender, marital status, and treatment utilization status.

Characteristic	N (%)	Mean
Age		65.1
50-60	4 (44.4%)	
61-70	2 (22.2%)	
71-80	3 (33.3%)	
Race/Ethnicity		
Black/African American	1 (11.1%)	
Non-Hispanic White	5 (55.6%)	
Hispanic	3 (33.3%)	
Gender		
Female	7 (77.9%)	
Male	2 (22.2)%	
Marital Status		
Married	3 (33.3%)	
Divorced	4(44.4%)	
Widowed	1 (11.1%)	
Single/Never Married	1 (11.1%)	
Education Level		
Some College	4 (44.4%)	
Bachelor's Degree or Higher	5 (55.6%)	
Mental Health Treatment		
Never	4 (44.4%)	
Past 1-5 Months	3 (33.3%)	
Past 6-11 Months	0 (0%)	
Over 1+Years Ago	2 (22.2%)	
Individuals in Household		
1	5 (55.6%)	
2	4 (44.4%)	
3+	0 (0%)	

Table 1. Respondent Characteristics.

Semi-structured interview

Interviews were conducted with all the participants (N= 9) at the local AAA in a small conference room. Interviews lasted approximately 30 minutes and followed a semi-structured interview format. The use of open-ended questions allowed for a broad and detailed description of participants' experiences with mental health service utilization and stigma. To assist in the structure of the interview, additional probing questions were asked to elicit further in-depth responses if necessary. Examples of questions asked included: "Can you tell me when you experienced a mental health disorder?"; "Can you tell me about some things that encouraged and discouraged you to seek treatment?"; "Can you describe a time in which you felt isolated, or stopped participating in certain activities because of your mental illness?"; and "Have you ever felt mistreated because of your mental illness, describe what happened?" Questions were designed to cater to the older adult population and to ensure comprehension of all questions asked. To show appreciation for their assistance in the study, participants received a monetary compensation of $25.00 after completion of the interview.

Data Analysis

Qualitative techniques were utilized in efforts to attain detailed description and give a voice to older adults with a co-occurring mental health diagnosis and to hear their unique experiences regarding the stigmatization of mental health conditions. This framework is used frequently throughout the field of Psychology. In this study, it will grant researchers with the opportunity to grasp the meanings, motives and patterns of perceived stigma in older adults with a co-occurring mental and physical condition that usually goes unnoticed in various standardized approaches, such as surveys.[27] To provide rich descriptions of the complex phenomena of stigma, thematic analysis was utilized to assess the data. The six-phase guide suggested by Braun and Clarke was used to successfully analyze the qualitative data.[25] Audio-recorded interviews were transcribed and re-read to ensure for accuracy. Printed versions of the transcriptions were read through thoroughly to familiarize ourselves with the data and noted for initial ideas. Subsequently the transcriptions were coded line by line, using the participants' words in attempt to summarize a detailed description of responses. The codes from each transcription were extracted from the data and collated into groups based on their similarities and categorized into potential themes.[25] The potential themes were examined for coherent patterns and refined to create overarching themes that best captured the context of the entire data set. A final codebook was created; vivid quotes were extracted from the transcriptions that best related to the literature and answered the research questions.[25] Despite a small sample size, saturation of the data was met with 9 interviews and at such time no further interviews were conducted.

RESULTS

As shown in **Table 1**, participants included 9 older adults (mean age of 65.1). The majority of the participants identified as Non-Hispanic White Females (55.6%) who were divorced (44.4%), received mental health treatment within the past year (77.8%) and lived by themselves (55.6%). All participants graduated from high school and attended at least some college, with some (55.6%) having a bachelor's degree or higher. As shown in **Table 2**, participants had one or more mental health conditions. Depression was the most prevalent (88.9%) mental health condition, followed by anxiety (22.2%), bi-polar disorder (11.1%), and schizophrenia (11.1%). All participants reported having one chronic physical illness. The most prevalent physical illness was diabetes (33.3%), followed by cancer (22.2 %) chronic back pain (22.2%), lupus (11.1%) and cardiovascular disease (11.1%).

Mental and Physical Health Condition	N (%)
Mental Health Condition	
Depression	8(88.9%)
Anxiety	2(22.2%)
Schizophrenia	1(11.1%)
Bi-Polar Disorder	1(11.1%)
Chronic Physical Illness	
Diabetes	3(33.3%)
Cancer	2(22.2%)
Chronic Back Pain	2(22.2%)
Lupus	1(11.1%)
Cardiovascular Disease	1(11.1%)

Table 2. Summary of Participants Health Status.

Interview Results

Results of the thematic analysis yielded four over-arching themes that aid in our understanding of the perceptions of stigma of older adults in recovery of a mental health diagnosis and living with a chronic physical illness. These themes included: 1.) Resilience from the Stigma of Mental Health Conditions, 2.) Community Engagement, 3.) Cultural Barriers and 4.) Social Support System. See **Table 3** for a description and overview of identified themes. Important quotes were extracted from the study participants' transcripts which best related to the identified themes. Study participants were given pseudonyms to protect their anonymity.

Resilience from the Stigma of Mental Illness
All participants reported living with stigma of a mental health condition and *not having someone to relate to, to confide in.* Participants' stigmatizing experiences were manifested in the forms of isolation, non-disclosure of mental health status and decreased help seeking behavior. When asked if they have ever felt mistreated from having depression**,** Sarah, a sixty-four-year-old, White woman stated, *"No because I didn't share it".* Some participants felt uncomfortable around their peers because of their mental health state. Ashley, a sixty-year-old white woman stated, *"…if this was not anonymous, you know if my name was given, I probably wouldn't want to give this information."*

Many participants were deterred from seeking professional treatment*, "I don't, I never thought that I needed it [treatment]…I would cry and that was it. I got it out of my system."* Some of the participants even began to accept the stigmatizing mistreatment from their peers. Grace, a sixty-year-old White woman stated, *"It [Depression] just seemed so engrained in a part of who I was it didn't seem like much that I needed to discuss or that I thought that it wasn't anything that anyone was able to fix."* She further stated, *"In these days when it happens I kind of laugh, depending on who it is. At the church where I go, this older man, he is the pastor. He learned what I have, and he says oh you're schizo, we had some people over the hill who were Schizo growing up, you are just schizo it is no big deal."*

Despite the mistreatment associated with their mental health condition, participants were more receptive toward disclosing their physical health status, but still *"felt isolated because of the pain and fatigue I was experiencing".* The co-occurrence of mental and physical conditions created some confusion for the participants. Sarah, a sixty-four-year-old, White woman stated she was unsure if the isolation was due to *"the physical manifestations of the lupus disease, or because of the depression… I have no idea how to sort those out".* Some participants even reported the stigma of their mental health condition surpassed them and affected their loved ones. It wasn't until the involvement of mental health organizations that allowed certain family members to accept the participants. Ashley, a sixty-year-old White woman stated: *"There has been a few family members, who didn't understand what it was, and my step-mom said you just need to buck up and you just need to deal with it, that kind of attitude. I had a brother-in-law that said I was being wimpy and all that stuff, but uhh he came around, cus he got hooked up with NAMI (National Alliance for Mental Illness)."*

When asked if they ever tried to reach out and confide in others, the participants did not know… *"who would I have talked to?"* Grace, a sixty-year-old White woman stated, *"No I couldn't of talked to her* [mother], *I was afraid of the ramifications, and isn't that sad. I really couldn't talk to anybody; I did tried to reach out to talk to friends, but not about this [Depression]."*Ashley, a sixty year-old White woman elaborated: *"Because of the stigma, and umm you know, even in our class, people don't understand it and you know they think you know, that there is a tendency for somebody to be violent and they just don't understand it you know. And you know they might think, you know who knows what they think".*

Some participants even reported effects of stigma in the form of mistreatment from their psychiatrists, where there were disagreements in treatment services. Sarah, a sixty-four-year-old, White woman elaborated: *"I went to the Psychiatrist twice…he did not tell me whether it would have an impact on the chemotherapy that allows me to keep my kidneys. So instead I got mad and went to the library and started researching, I did not take any medication".* Ashley, a sixty-year-old, White woman stated:
"I feel like being at a hospital when I was young, I guess being in the hospital and not being treated well, I feel like it had to be more than this. I feel like, that something was gonna come back you know, you know I would get something out of that because I was mistreated."

Despite the negative situations that the study participants experienced, those who acknowledged having a mental health condition were able to overcome the stigma. Participants were able to accept their mental health status and are more comfortable sharing their stories. When asked how their experience with a mental health condition changed over the years Sarah, a sixty-four-year-old White woman reported: *"Yeah I don't see myself as depressed anymore".* All the participants reported having better experiences with their mental health condition. Grace, a fifty-eight-year-old, white woman stated: *"It's gotten a lot better, especially since I've gotten the proper diagnosis. I was diagnosed with Bipolar disorder for a long time, it wasn't until I moved down here, that the true self was becoming about".* Andre, a sixty-year-old, African American man stated: *"My experiences have definitely become more positive. Umm I'm less likely now to ever want to harm myself or anyone else, you know I'm in a better place. Umm I wake up every morning and I feel great, I feel good, I feel energetic, I feel hey, I'm blessed to be blessed and I want to live you know".*

Participants reflected on their past negative experiences from living with a mental health condition and are now more comfortable sharing their stories. Having recovered from their co-occurring mental health diagnosis, participants wished *everybody knew at least about mental illness, we could let go of some of the stigma.* Gloria, a fifty-four-year-old white woman stated, *"I was too fragile to reach out to hardly anybody, and so now being more whole and having gone through process and found ways for self-care, it's easier to share that knowledge, because I have it, and I didn't have it back then."*

Community Engagement
One of the ways participants dealt with their experience with a mental health condition was giving back to the community. Many participants felt "*that something compels me to want to help others, and that is what I do, I want to help others*". Many participants emphasized how "*It is important to help other people to find a way to hold the light and help them find their way out."* The desire to give back to community was a recurrent ideology in the study. Ashley, a sixty year-old, White woman stated: "*It is just better, I feel like I'm dealing with it you know, and that was one thing I was attracted to this program, because you know I feel like I could help people because I've been through it myself and I'm older and umm I have come to terms with it ."*

Participants enjoyed the personal benefit from being able to share their stories with others. Participants strongly suggested "*the elderly people need that kind of help.*" The need to provide emotional support and advice to their peers who are currently experiencing a mental health condition was a pertinent concern amongst the study participants. Andre, a sixty-year old, African American man stated: "*It makes me feel good when someone else listens to me and I tell them that um some of my experiences and they ask me well how did you do it*". Many participants were eager to provide services so others "*don't have to go through what I went through 40 years ago, you know not having someone to relate to, to confide in.* Ashley, a sixty-year-old, White woman stated: "*I guess my main idea is trying to benefit the other person you know and I guess when, if I am able to do that you know, I would like to see them being healthy and um out of the hospital*".

All participants had some form of higher education beyond high school and felt that their education and past *"experiences working with the elders"* made them especially qualified to help others. Sarah, a sixty-four year-old, White woman stated, *"I am already a master trainer for Stanford University chronic disease self-management program, and as program director for the Lupus Foundation, I deal with patients who are in often depressed, and don't know where to turn or can't figure out the solutions having being in that situation, it is important to help other people find a way to hold the light and to help them find their way out"*. Victor, a seventy-eight-year-old, Hispanic man stated: *"At USF, I went to Stanford to take CDS and PD, it's a training to help people with chronic illnesses and management problems. Uh you coach that, and it was a good experience you know, the elderly people here they need that kind of help, so we went around and talked about chronic diseases and how to manage them and the proper thing and mind and that thing"*.

All participants of the study reported positive experiences engaging with members of their community who were in the process of recovering from a mental health condition. For many of the participants, involvement in these social programs became a significant part of their everyday life. In addition, many participants even reported receiving appropriate certification and training, allowing them to be better equipped to service their peers.

Cultural Barriers
Culture and cultural barriers appeared to play a very important role in participants' perceptions of stigma and seeking mental health treatment. It also played a role in how participants verbalized their experience of having a mental health condition. All the study participants who self-identified as Hispanic (N= 3) were uncomfortable to openly acknowledge the presence or history of having a mental health condition. When asked to describe a time when they experienced a mental health condition they responded with "*I've never experienced a mental health disorder*". However, when asked if they have experienced recurrent episodes of intense feelings of sadness, or other symptoms commonly associated with a mental health diagnosis, the study participants responded and had unique stories to share. Lisa, a sixty-three-year-old Hispanic woman stated: *"No I feel like no one can help me really. You know cus I get depressed sometimes, because like I said my family is very far away or gone away, or so I just keep things to myself"*.

Participants felt that the experience of symptoms of depression was a normal experience, something that everyone goes through. Participants felt they needed to justify not being depressed. For example, one participant stated she wasn't "*depressed I would call it more like I was bored, and I felt that I needed to do something with my life*". Sasha, a seventy-four-year-old, Hispanic woman said, "*It's not something that I carry with me at all, it is just something that I consider uh normal, you know*". Lisa, a sixty-three year old, Hispanic woman stated: *"You know I have even heard umm, even in Spanish they say 'If you are gonna cry, cry in your house, when you are alone and nobody sees you or whatever, and then you get over it', or so you don't have to you know, show your emotions that much, you're always being what you don't feel like being."*

Culture also played a role in the mental health services individuals engaged in. While all participants identified themselves as in recovery from a mental health condition, participants did not all choose formal professional mental health services as an avenue for treatment. Participants identified themselves as "*very family oriented, so no the idea, the thought never crossed my mind. I didn't need it. I had all the support that I needed."* Participants found various non-clinical ways to cope with their mental health conditions. Participants often looked to family for social support, participated in other activities as an escape mechanism, engaged in community-based activities where they could give to others, or were just content with their emotional state and pushed through it on their own. Victor, a seventy-eight-year-old, Hispanic man stated: *"I kind of adapted through it, you know I've come to the conclusion that it is a part of my life, you now I have to deal with it and umm it doesn't bother me you know."*

Theme w/ Definition	Direct Quote
Cultural Barriers Cultural customs and beliefs that inhibit individuals from seeking professional treatment and disclosure of their mental illness.	*PE7: I always look at the positive things, I believe very much in the power of mine, so you got to be positive. You know whatever happens it happens, it is what it is and that's it.* *PE6: I don't know if you know about Cubans, but we're like very family oriented, so no, the idea [mental health treatment], the thought never crossed my mind. I didn't need it, I had all the support that I needed.*
Benefit of Social Support The need to empathize and sympathize with others about one's problems regarding their mental health.	*PE2: I had a brother-in-law that said I was being wimpy and all that stuff, but uhh he came around, cus he got hooked up with NAMI. Somehow he got hooked up, oh my nephew got addicted to drugs, and tried to commit suicide, and that took them on a different journey then they were used to, and so through that he got hooked up with NAMI and learned about addictions and learned about different stuff, and he ended up apologizing to me for picking on me for so many years. He finally realized it wasn't my fault, so that was nice…* *PE4: No in fact, I do think that one of my art teachers, she keyed on to the fact that I was having trouble with life. I think a lot of the teachers in high school knew that there was a lot going on with me, then what was showing on the surface, and she used to do things, like she brought me in a necklace that she macro-made for me… But if anything, I'd have to say that they had a hand around my shoulder because they wanted to*
Resilience from the Stigma of Mental Illness The ability to overcome the barriers associated with having a mental health condition, and work toward a state of recovery.	**Past Experiences:** *PE1: I had acute onset of systemic lupus that damaged most of my organs; my heart, lungs, brain, blood, bone-marrow and kidneys. I had to have a kidney biopsy, I had to go on chemotherapy for life, I lost both of my jobs, used up my entire pension and I was living in poverty and at that point I didn't have disability yet and there was an eviction notice on my door and I got three months of food stamps and that was all I had. It was serious depression where I had suicidal ideations and sought treatment….* *PE2: I started having panic attacks in high school…I moved down here in Florida in '82 and the panic attacks got worse, and they ended up giving me Valium... But I was married to an abusive alcoholic husband…There has been a few family members, who didn't understand what it was and my step-mom said you just need to buck up and **you just** need to deal with it, that kind of attitude. I had a brother-in-law that said I was being wimpy.* *PE3: I was never officially diagnosed, I would never, my father never approved of it, and I always did what my father said…I knew that something was wrong and that we needed to talk about it, but that is what they didn't do. No, you didn't talk about it, you just let it lay there, so yeah.* **Current Experiences:** *PE4: In these days when it happens I kind of laugh, depending on who it is. At the church where I go, this older man, he is the pastor. He learned what I have and he days oh you're schizo, we had some people over the hill who were Schizo growing up, you are just schizo it is no big deal* *PE2: It's gotten a lot better, especially since I've gotten the proper diagnosis. I was diagnosed with Bi-Polar disorder for a long time, it wasn't until I moved down here, that the true self was becoming about* *PE3: Umm yesss better [experience with a mental health condition] …um I think it was when I went to high school, it was either when I went to high school or when I went to college. Umm I went to this class in psychology, it was like an opening and umm then I realized what my sister was doing to me, and umm I'm not going to do this anymore and try to do it, and umm okay she made me feel less than an ant okay, and she okay can make me feel miserable. And umm okay I have been free for three years, Hallelujah! …*
Community Engagement The importance of giving back to society and helping others with a mental illness who are currently experiencing similar struggles.	*PE 6: I figured if I can be a help to someone, you know I think on a very selfish way, I'm going to feel good about it to. It's always like that, you know you have no idea, you do something for someone and amazingly so, sometime you are the recipient of that good feeling, because you feel good about it, so it is a combination of things, that you know I don't know if I'm going to be good at it, but that is why I am interested. I think my experience with the elders have been so good, that I think if I can help someone, I should, I think I should.* *PE7: …the elderly people here they need that kind of help, so we went around and talked about chronic diseases and how to manage them and the proper thing and mind and that thing. You know I got into matter of balance here, and I realized that it does help people, you know they feel every time we coach a course, they come back and say hey how grateful they are, and that's a self-reflection.*

Table 3. Sample Responses to the Semi-Structured Interviews.

Social Support System
All of the study participants stressed the importance of having individuals to reach out to in order to cope with their mental health condition. Lisa, a sixty-three year old Hispanic woman stated, *"…she [my sister] give me a lot of advice and cause she was, she was married at one time and she left her husband, and she divorced him and she went back to live with my mom and so I rely on her, because she went through what I was gonna go through again"*. Gloria, a fifty-four year-old, White woman stated: *"So everything, came crashing down at that point and I remember calling my sister in the middle of the night, saying I need to go somewhere, because I'm going to hurt my kids or something, I need to get out of here, so somehow I ended up at her house and she just took me to a psychiatrist"*.

Some participants wanted someone who knew what they were going through in order to overcome a mental health condition. Participants shared stories on how others were unable to understand the extent of their mental health condition. Sarah, a sixty-four year-old, White woman elaborated, *"I don't think they had the skill or the knowledge or that they* [her children] *were the right person, the right person to help, because they wouldn't of had that understanding and then I was afraid that they would capture me and make me live with them"*. Grace, a fifty-eight year- old, White woman stated: *"…you never really know the pain anybody else is going through, umm to be able to walk with them and to be able to empathize rather than sympathize, because you, you know what it's like to have a mental illness yourself"*.

Some participants even talked about the benefits of engaging in support groups for individuals currently experiencing a mental health condition. Andre, a sixty year-old, African American man stated: *"People don't think groups work, groups help when you share with other people, AA groups help, if you want to stop smoking cigarettes, nicotine groups help if you want you stop using drugs, there are drugs groups that help"*. Ashley, a sixty-year-old, White woman stated: *"The BDSA the Bipolar alliance, they have groups and those are great if someone is able to go out and umm be a part of those groups. I know those really help, not to feel isolated when you have umm bi-polar, because that umm a support group just for umm people that have mental illness"*.

Social support was an important factor in the process of recovering from a co-occurring mental health diagnosis. For many of the participants, the ability to avoid judgment when conveying their thoughts and feeling to others was essential in their path toward recovery.

DISCUSSION

This study provides critical information on the unique experiences of stigma among older adults who are in recovery from a mental health condition who are currently living with a chronic physical illness. These anecdotal recollections of participants' stigma associated their health status, will help to get a better understanding of the issues associated with mental health treatment utilization. The semi-structured interviews elicited four overarching themes regarding their experiences. Findings in the study echo results in presented in the current literature. Cultural beliefs play a major role in individual perceptions of mental health conditions. Due to the stigma associated with the label of mental health conditions, many Hispanic individuals refer to their mental health condition as mental distress; this term is more congruent with the Hispanic cultural beliefs and ideologies about mental health conditions.[28] One of the most crucial and challenging stages in recovery of a mental health condition is acceptance. During this stage individuals must overcome their negative self-judgments, develop a positive identity and truly understand the extent of their mental illness.[29] Hispanic individuals rarely accept the diagnosis of mental health conditions because their perceptions of its etiology often differ from Non-Hispanic individuals. Research shows that the Hispanic communities attribute mental distress to negative childhood experiences, acculturative stress, and relationship and family problems.[28] A study conducted by Thoman and Suris found that acculturative stress experienced by Hispanic individuals was a significant predictor for psychological distress in comparison to individuals who have not experienced acculturating situations.[30]

Although the study highlighted the cultural barriers faced in Hispanic communities, other cultures attribute the etiology of mental health conditions to distinct factors. For example, in the African American community many individuals justify mental health conditions being caused by super-natural forces.[28] Studies have also reported high-levels of stigma in the Latino population as a significant predicting factor toward unwillingness to seek treatment.[31] It is important for mental health clinicians to implement culturally sensitive diagnostic measures and interventions when assessing mental health conditions in individuals from differing cultural backgrounds. Implementing culturally appropriate services will positively impact the under-diagnosis and underutilization of treatment service disparities in the minority population of older adults with a mental health condition.

Participants in the present study all identified unique past experiences where they were mistreated by their friends and family because of the status of their mental health. Stigma can be exhibited from various instances of mistreatment including: the refusal to disclose mental health status, lack of social support from their loved ones, and isolation from members in their community.[32] Participants of the study, who shared their mental health state to others without a mental health condition, reported perceived lack of understanding and sympathy from the other party. Families and friends of individuals with a mental health condition are also affected by stigma, resulting in their overt act of judgment and ostracism of persons with a mental health condition.[32]

Many participants were reluctant to seek professional mental health treatment. Stigma associated with a mental health condition is known to perpetuate a cycle of disability, halting patient's process of recovery.[32] Older adults who live in communities who perceive them as "mentally ill" are often more stigmatized than individuals who have other non-mental health conditions[33]. Society often produces stigmatizing reactions toward older adults who have a mental health condition because of their eccentric psychiatric symptoms, social-skill deficits and physical appearances.[33] In addition, a few of the study participants with acute physical illnesses, neglected to utilize appropriate medical interventions. Failure to disclose physical health complications to the respected clinicians is a frequent occurrence in individuals with a mental health condition.[41, 42] Furthermore, physical health complications of people with mental health conditions are often neglected by clinicians, resulting in increased mortality rates

compared to people with solely physical illnesses.[41, 42] Individuals with various mental health conditions are not receiving equitable treatment and tend to receive treatment for only life-threatening diseases.[41, 43] The neglect of clinicians' non- probing behaviors and individual's non-disclosure of their physical health state can be attributed to the stigma of having a mental health condition.[41-43] Therefore, the stigma associated with having a mental health condition needs to be addressed across all clinical settings in efforts to reduce the mortality of people with both mental and physical health complications and to provide for a more inclusive environment.

In the current study, all the Hispanic participants refused to disclose their mental health status, due to fear of the negative consequences associated with having a mental health condition. Studies suggest that labeling is the most significant manifestation of stigma.[33] Labeling often develops into stereotypical beliefs in which members of a community categorize individuals with a mental health condition as being violent, incompetent and responsible for the onset and continuation of their disorder.[33] A study conducted by Sirey and colleagues, suggested that age is a significant predicting factor regarding the perception of stigma.[24] The study suggested that adults (under 65) are more likely to perceive more stigmas about a mental health condition than older adults.[24] Older adults that are aware of the manifestations of stigma often alter their behavior accordingly and avoid the mistreatment and discrimination from members in their communities. Additionally, the Hispanic and African American participants, reported solace from their spiritual and religious practices. Cultural practices are often used in times of physical and mental distress, to increase an individual's quality of life.[44, 45] Research suggest that religiosity encourages safer coping mechanisms, enhances pain management, reduces the risk of suicide, improves surgical outcomes and reduces the onset of depression.[44, 45] It is imperative for clinicians to utilize a holistic treatment approach to combat the effects of stigma on treatment utilization.

A few of the participants in the current study with past stigmatizing experiences were able to adapt to the adversity of a mental health condition through a supportive network of peers, family and friends. Research shows that increased help seeking behavior from professionals or peers is associated with increased resilience to a mental health condition and decreased stigma.[34] Individuals become resilient to a mental health condition when they have encountered an experience that granted them with the opportunity needed to overcome stigma; are able to share their experiences of stigma with others; accept the status of their mental health condition, have faith and form meaningful relationships.[34, 35] Due to individuals' beliefs of stigma potentially inhibiting treatment seeking behaviors, it is important that mental health clinicians become aware of the role resiliency plays in seeking professional help. In order to increase the number of individuals seeking mental health treatment, it is imperative to educate the public, those who do and do not currently need mental health treatment about the benefits of mental health treatment.[34] If individuals with a mental health condition live in a society where treatment for mental health conditions are encouraged rather than stigmatized, it may decrease the large population of undiagnosed, and untreated older adults with a mental health condition.

In the current study, participants who carried these resiliency characteristics were all enrolled in a community engaging project as peer educators. This project created opportunities for them to help others suffering from similar problems. Participation in these community engagement activities allow older adults to learn more about other people and share their values with others, causing them to have increased sense of well-being.[36] Individuals who have a history of utilizing various mental health treatments gain unique and potentially valuable insights from their treatment experiences, in which they share with appropriate personnel to improve the provision of mental health services.[37-39] Many older adults in recovery of a mental health condition have made use of their life experiences, problem-solving skills, social skills and formal training to promote independent functioning and social integration amongst their peers who are currently experiencing a mental illness.[40] Research suggests that older adult's participation in social engagement interventions lead to an increased quality of life, have enhanced self-esteem, self-confidence and self-efficacy.[40] Older adults who have participated in these programs have worked toward reducing the stigma of having a mental illness, through empathizing and educating others dealing with mental health related issues.[40] It is important for mental health clinicians to encourage older adults to participate in these activities to improve their quality of life. It is important to note that there are limitations that need to be identified. The research incorporated a small sample of nine participants from the same geographical location. The sample also lacked diversity. This sample consisted of highly educated individuals, which may have affected their knowledge about mental illnesses and their willingness to seek treatment. As a result, findings from the current study cannot be generalized. Despite these limitations, this is one of the only studies which have qualitatively examined perceptions of stigma and experiences of seeking professional mental health services from a vulnerable sample of older adults in recovery from a mental health condition who are currently living with a chronic physical illness.

CONCLUSIONS

The stigma of mental illness is a pertinent issue among older adults living with a co-occurring mental health diagnosis. Findings from the current study suggest the need for mental health services catering to our rapidly growing population of older adults. Specifically, health care professionals need to develop solutions to decrease the large number of older adults going undiagnosed and untreated for a mental health condition. Additionally, to avoid stigmatizing experiences from having a mental health condition, more programs educating persons about mental health conditions need to be implemented nation-wide. Due to the evident cultural barriers influencing the treatment utilization behaviors of racial and ethnic minorities, it is important to allocate funding for older adults to service in lay health educator roles. Further research is needed to examine the perceptions of stigma among populations of older adults who have been able to overcome the barriers of stigma, but are not affiliated with community engaging programs, and older adults with different physical conditions. This will allow for more unique resilient strategies to be recreated and taught to other elders in current psychiatric distress.

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PRESS SUMMARY

Older adults are a vulnerable population who are more susceptible to developing mental health conditions, and the symptoms are often made worse by the co-occurrence of various physical health complications. The present study provides a deeper understanding of the stigmatizing obstacles this population has overcome in their journey toward recovery of a mental health condition.