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Application of statistical contrasts to mean refractive state

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Abstract

Testing for differences between specific groups or combinations of groups is referred to as comparison or contrast testing. Statistical significance of comparisons can be assessed by first forming contrasts and then testing for their significance. A contrast essentially tests whether or not two means are significantly different, where each mean could be a weighted average of two or more means.

Gillan investigated whether the instillation of a cycloplegic (Mydriacyl 1% (tropicamide)) into the right eye of a 30-year-old female subject would affect the variability of her distance refractive state as measured by an autorefractor.

The purpose of this paper is to introduce mutually orthogonal linear contrasts of sample data to optometric research. By constructing particular contrasts, mean refractive states are compared before instillation, before and after instillation, and after instillation.

Keywords: Multiple comparisons, contrasts, hypothesis testing.

Introduction

When measuring refractive state using an autorefractor, different types of refractive variation have been found. Repeated measurements of refractive state reveal variability of the refraction. Gillan[1] considered refractive variability under cycloplegia in a 30-year-old female subject. He analysed the experimental data by means of multivariate statistical methods developed by Harris[2] and used software developed by Harris and Malan. A shortfall of Gillan[1] is that the null hypothesis and the alternate hypothesis for the various stages of the experiment were neither stated nor justified when he performed the different hypotheses tests on the sample data. Since he only performed the experimental procedure on a single subject, conclusions can only be made about the mean refractive state of that subject. However if the same experimental procedure was performed on the right eyes of two or more 30-year-old female subjects selected at random from a population of 30-year-old females, then one could make conclusions about the mean refractive state of the population of right eyes of 30-year-old females using the sample data. Malan[3] questioned statistical analysis of refractive variability with small samples.

Mutually orthogonal linear contrasts for comparing J multivariate means require J – 1 such contrasts. Using the theory of mutually orthogonal linear contrasts[4], Lemmer[5] discussed only a single contrast as being an optimal way to ascertain whether the cycloplegic had a significant effect. One queries the validity of this approach, as he neglected the mutually orthogonal requirement. References [6-10] were also consulted for discussion of contrasts and multiple comparisons.

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This paper applies mutually orthogonal linear contrasts to sample data to investigate whether the instillation of a cycloplegic into the right eye of a 30-year-old female subject would affect the variability of her distance refractive state as measured by an autorefractor. Data modified from an experiment designed and carried out by Gillan[1] are used. Details of the experimental procedure are available[1], but are not considered necessary in so far as this paper is concerned.

Hypotheses about contrasts

In order for any tests of hypotheses or rules of decisions to be good, they must be designed so as to minimize errors of decisions. This is not a simple matter since, for a given sample size, an attempt to decrease one type of error is accompanied in general by an increase in the other type of error. In practice one type of error may be more serious than the other, and so a compromise should be reached in favour of a limitation of the more serious error. The only way to reduce both types of error is to increase the sample size, which may or may not be possible.

Testing for differences between specific groups or combinations of groups is referred to as comparison or contrast testing. Statistical significance testing of comparisons can be assessed by first forming contrasts and then testing for their significance. It is a good statistical practice to perform contrast analysis determined or stated *a priori*, rather than test all possible contrasts in search of significant effects. In univariate significance tests, each contrast is tested separately for each dependent variable, whereas in multivariate significance tests, each contrast is tested simultaneously for all the dependent variables. Mutually orthogonal linear contrasts are applied to data as published by Gillan[1]. The raw data were not available and minor errors in Gillan's tables were corrected (a negative variance in Gillan's Table 2 was corrected to its positive value. See Table 1 and Table 2 modified from Gillan[1]).

Table 1: The mean refractive state for each set of data measured by Gillan[1] is indicated. Sample size is the same (n = 30) in each case. BM1: Data collected 30 minutes prior to instillation of Mydriacyl into the right eye of the subject, BM2: Data collected immediately prior to instillation, AM1: Data collected 15 minutes after instillation, AM2: Data collected 30 minutes after instillation and AM3: Data collected 60 minutes after instillation. All quantities have units D, except for axis which is measured in degrees.

	Mean refractive state (conventional form)			Mean refractive state as vector h		
	sph	*cyl*	*axis*	h_1	h_2	h_3
BM1	–0.0377	–0.1407	x 157	–0.0582	–0.0702	–0.1578
BM2	0.0103	–0.1516	x 161	–0.0049	–0.0644	–0.1261
AM1	–0.0245	–0.1646	x 158	–0.0476	–0.0808	–0.1661
AM2	–0.0513	–0.1618	x 159	–0.0716	–0.0759	–0.1927
AM3	–0.0534	–0.1768	x 162	–0.0693	–0.0715	–0.2143

Table 2: Data modified from Gillan[1]. The variance-covariance matrix for vector h is shown for each data set collected. BM1: Data collected 30 minutes prior to instillation of Mydriacyl into the right eye of the subject, BM2: Data collected just prior to instillation, AM1: Data collected 15 minutes post instillation, AM2: Data collected 30 minutes post instillation and AM3: Data collected 60 minutes post instillation. All quantities have units D^2.

BM1	0.00196 0.00045 0.00082 0.00045 0.00078 0.00003 0.00082 0.00003 0.00129
BM2	0.00213 –0.00042 0.00129 –0.00042 0.00094 –0.00017 0.00129 –0.00017 0.00348
AM1	0.00191 –0.00100 0.00180 –0.00100 0.00117 –0.00092 0.00180 –0.00092 0.00328
AM2	0.00170 –0.00118 0.00175 –0.00118 0.00191 –0.00113 0.00175 –0.00113 0.00293
AM3	0.00208 –0.00037 0.00201 –0.00037 0.00103 –0.00074 0.00201 –0.00074 0.00419

Univariate significance tests for the contrasts

A contrast among J population means μ_J is a weighted sum

$$c_1 \mu_1 + c_2 \mu_2 + \ldots + c_J \mu_J$$

where $c_j \neq 0$ for some j , and $c_1 + c_2 + \ldots + c_J = 0$. This is also referred to as a linear contrast. Linear contrasts make comparisons amongst the J means $\mu_1, \mu_2, \ldots, \mu_J$. A contrast is denoted by ψ which may be subscripted if the experimenter considers more than one set of weights

$c_1, c_2,\ldots, c_J$. Thus, some of the contrasts we might define for five populations are:

$\psi_1 = (0)\ \mu_1 + (2)\ \mu_2 + (1)\ \mu_3 + (0)\ \mu_4 + (-3)\ \mu_5$

$\psi_2 = (1)\ \mu_1 + (2)\ \mu_2 + (3)\ \mu_3 + (6)\ \mu_4 + (-12)\ \mu_5$

$\psi_3 = (0)\ \mu_1 + (0)\ \mu_2 + (0)\ \mu_3 + (1)\ \mu_4 + (-1)\ \mu_5$

$\psi_4 = (1/2)\ \mu_1 + (1/2)\ \mu_2 + (0)\ \mu_3 + (0)\ \mu_4 + (-1)\ \mu_5$

The hypothesis $\psi_3 = 0$ is equivalent to the hypothesis that $\mu_4 - \mu_5 = 0$, that is $\mu_4 = \mu_5$. Likewise, ψ_4 can be rewritten $\psi_4 = (\mu_1 + \mu_2)/2 - \mu_5$, so that the hypothesis $\psi_4 = 0$ is equivalent to the hypothesis that $(\mu_1 + \mu_2)/2 - \mu_5 = 0$, that is, the hypothesis that the average of μ_1 and μ_2 is equal to μ_5.

In general, any such comparison among J population means can therefore be formulated in terms of a test hypothesis about a contrast:

$H_0 : c_1 \mu_1 + c_2 \mu_2 + \ldots + c_J \mu_J = 0$

or

$H_0 : \psi = 0.$

The alternative hypothesis is

$H_1 : \psi \neq 0$

Let $A = a_1 \mu_1 + a_2 \mu_2 + \ldots + a_J \mu_J$ and $B = b_1 \mu_1 + b_2 \mu_2 + \ldots + b_J \mu_J$ be two linear contrasts of the means $\mu_1, \mu_2,\ldots, \mu_J$. Then A and B are called orthogonal linear contrasts if in addition to:

$a_1 + a_2 + \ldots + a_J = 0$ and $b_1 + b_2 + \ldots + b_J = 0$,

it is also true that:

$a_1 b_1 + a_2 b_2 + a_3 b_3 + \ldots + a_J b_J = 0.$

None of the contrasts ψ_1 to ψ_4 defined above for the five populations is orthogonal.Orthogonal linear contrasts make independent comparisons amongst the J means $\mu_1, \mu_2,\ldots, \mu_J$.

Let $A = a_1 \mu_1 + a_2 \mu_2 + \ldots + a_J \mu_J$,
$B = b_1 \mu_1 + b_2 \mu_2 + \ldots + b_J \mu_J$, and
$L = l_1 \mu_1 + l_2 \mu_2 + \ldots + l_J \mu_J$
be a set of linear contrasts of the means $\mu_1, \mu_2,\ldots, \mu_J$. Then the set is called a set of mutually orthogonal linear contrasts if each linear contrast in the set is orthogonal to any other linear contrast in the set. The maximum number of linear contrasts in a set of mutually orthogonal linear contrasts of the means
$\mu_1, \mu_2,\ldots, \mu_J$ is J – 1. The number of degrees of freedom for comparing the means

$\mu_1, \mu_2,\ldots, \mu_J$ is J – 1.

Let $L_1, L_2, \ldots, L_{J-1}$ denote J – 1 mutually orthogonal linear contrasts for comparing the J means $\mu_1, \mu_2,\ldots, \mu_J$. Then the sum of squares for comparing the J means based on J degrees of freedom, $SS_{Between}$, satisfies:

$SS_{Between} = SS_{L_1} + SS_{L_1} + \ldots + SS_{L_{J-1.}}$

Defining a set of mutually orthogonal linear contrasts for comparing the J means $\mu_1, \mu_2,\ldots, \mu_J$ allows the researcher to break up the sum of squares for comparing the J means, $SS_{Between}$, and perform individual tests of each linear contrast.

Suppose $c_1 + c_2 + \ldots + c_J \neq 0$, and we want to use the theory of linear contrasts for the testing procedure. Suppose that the coefficients c_1 and c_2 are zero, but that $c_3, c_4, .., c_J$ are nonzero. Compute the mean of those coefficients that are nonzero, that is calculate $\bar{c} = \frac{c_3 + c_4 + \ldots + c_J}{J - 2}$.
Then $(c_3 - \bar{c}) + (c_4 - \bar{c}) + \ldots + (c_J - \bar{c}) = 0$,
that is, we have a linear contrast with
$\hat{c}_3 = c_3 - \bar{c}$, $\hat{c}_4 = c_4 - \bar{c}$, ..., $\hat{c}_J = c_J - \bar{c}$ as the new coefficients. Of course one must also satisfy the requirement that the contrasts be mutually orthogonal.

Multivariate significance tests for contrasts

Multivariate contrasts are used to test simul-

taneously for the effects of all the dependent variables. A multivariate contrast is given by

$$\boldsymbol{\Psi}_i = \mathbf{c}_{i1}\,\boldsymbol{\mu}_1 + c_{i2}\,\boldsymbol{\mu}_2 + \ldots + \mathbf{c}_{iJ}\,\boldsymbol{\mu}_J$$

where $\boldsymbol{\mu}_J$ is a vector of means for the J-th group and $\boldsymbol{\Psi}_i$ is the i-th contrast vector.
The null and alternate hypotheses for the multivariate significance test for the i-th contrast are

$$H_0 : \boldsymbol{\Psi}_i = \mathbf{0} \quad \text{and} \quad H_1 : \boldsymbol{\Psi}_i \neq 0.$$

According to Sharma[4] the test statistic for H_0 is given by

$$F = T^2 (df_e - p + 1) / (df_e \,.p) \qquad (1)$$

where $T^2 = \left(\sum_{k=1}^{G} \frac{c_{ik}^2}{n_k} \right)^{-1} \boldsymbol{\Psi}_i' \; \mathbf{S}_w^{-1} \; \boldsymbol{\Psi}_i$

with n_k the number of observations in each group k, G the number of groups, p the dimension of the observations, $\mathbf{S}_w$ the pooled within-group covariance matrix with df_e degrees of freedom. The F-statistic has an F-distribution with p and $df_e - p + 1$ degrees of freedom.

Example

Mean refractive state – testing the data from Table 1.

Let $\boldsymbol{\mu}_1$ be the mean refractive state of the right eye of a 30-year-old female subject 30 minutes before the instillation of Mydriacyl, $\boldsymbol{\mu}_2$ the mean refractive state of the eye just prior to instillation, $\boldsymbol{\mu}_3$, $\boldsymbol{\mu}_4$ and $\boldsymbol{\mu}_5$ the mean refractive state of the eye 15 minutes, 30 minutes and 60 minutes respectively post instillation.

Multivariate statistical methods developed by Harris[2], as well as mutually orthogonal linear contrasts discussed by Sharma[4] are implemented and compared where possible. Hypotheses tests are performed at a 5% and at a 1% level of significance. All the numerical computations are performed using Matlab.

(i) Using the method of Harris[2], two possible starting reference values namely $\boldsymbol{\mu}_1$ and $\boldsymbol{\mu}_2$ are available for performing hypotheses tests. In *A1* to *A4*, $\boldsymbol{\mu}_2$ is used as reference value, (same as Gillan[1]), while in *B1* to *B4* , $\boldsymbol{\mu}_1$ is used as reference value. Note that the tests *A1* and *B1* are identical.

A1	$H_0: \boldsymbol{\mu}_2 = \boldsymbol{\mu}_1$ $H_1: \boldsymbol{\mu}_2 \neq \boldsymbol{\mu}_1$	*A2*	$H_0: \boldsymbol{\mu}_2 = \boldsymbol{\mu}_3$ $H_1: \boldsymbol{\mu}_2 \neq \boldsymbol{\mu}_3$
A3	$H_0: \boldsymbol{\mu}_2 = \boldsymbol{\mu}_4$ $H_1: \boldsymbol{\mu}_2 \neq \boldsymbol{\mu}_4$	*A4*	$H_0: \boldsymbol{\mu}_2 = \boldsymbol{\mu}_5$ $H_1: \boldsymbol{\mu}_2 \neq \boldsymbol{\mu}_5$
B1	$H_0: \boldsymbol{\mu}_1 = \boldsymbol{\mu}_2$ $H_1: \boldsymbol{\mu}_1 \neq \boldsymbol{\mu}_2$	*B2*	$H_0: \boldsymbol{\mu}_1 = \boldsymbol{\mu}_3$ $H_1: \boldsymbol{\mu}_1 \neq \boldsymbol{\mu}_3$
B3	$H_0: \boldsymbol{\mu}_1 = \boldsymbol{\mu}_4$ $H_1: \boldsymbol{\mu}_1 \neq \boldsymbol{\mu}_4$	*B4*	$H_0: \boldsymbol{\mu}_1 = \boldsymbol{\mu}_5$ $H_1: \boldsymbol{\mu}_1 \neq \boldsymbol{\mu}_5$

Table 3: Test statistics for hypotheses tests *B1* to *B4* on mean refractive state using μ_1 as reference value, as well as test statistics for hypotheses tests *A1* to *A4* on mean refractive state using μ_2 as reference value (same as Gillan[1]). The respective null hypotheses (H_0) are that the mean refractive states are equal. Critical values are $F_{0.05,\,3,\,56} = 2.776$, and $F_{0.01,\,3,\,56} = 4.166$. Note that the tests *B1* and *A1* are identical.

Hypothesis Test	Test statistic using equation 22 [2], with decision on H_0 denoted by * and **
B1 and *A1*	6.9286 * and **
B2	1.8848
B3	3.9248 *
B4	7.6609* and **
A2	10.3910* and **
A3	18.5603 * and **
A4	14.6503 * and **

* Reject H_0 at 5% level of significance.
** Reject H_0 at 1% level of significance.
No asterisks: Retain H_0 at the appropriate levels of significance.

Gillan[1] only states results using $\boldsymbol{\mu}_2$ as reference value. However, no values are displayed. Lemmer[5] questions what would have resulted had $\boldsymbol{\mu}_1$ been used as reference value. The results obtained with both reference values $\boldsymbol{\mu}_1$ and $\boldsymbol{\mu}_2$ are shown in Table 3.

Lemmer[5] states that it is not good statistical practice to test each of the after treatments in turn against the second set of observations because the Type I error is thereby enlarged. If

Table 4: A choice of 4 mutually orthogonal linear contrasts for the 5 means $\mu_1, \mu_2, \mu_3, \mu_4, \mu_5$.						
Contrast coefficients $c_i \rightarrow$	c_1	c_2	c_3	c_4	c_5	**Sum across rows**
Contrast 1 (C1)	1	–1	0	0	0	0
Contrast 2 (C2)	1/2	1/2	–1/3	–1/3	–1/3	0
Contrast 3 (C3)	0	0	1	–1	0	0
Contrast 4 (C4)	0	0	1/2	1/2	–1	0
Product of c_i's in contrast columns						**Sum across rows**
C1 & C2	1/2	– 1/2	0	0	0	0
C1 & C3	0	0	0	0	0	0
C1 & C4	0	0	0	0	0	0
C2 & C3	0	0	–1/3	1/3	0	0
C2 & C4	0	0	–1/6	–1/6	1/3	0
C3 & C4	0	0	1/2	–1/2	0	0

Table 4a: Mutually orthogonal linear contrasts and corresponding null hypotheses.	
Contrast	**Null hypotheses H_0**
Contrast 1 (C1)	$\mu_1 - \mu_2 = 0$
Contrast 2 (C2)	$(\mu_1 + \mu_2)/2 - (\mu_3 + \mu_4 + \mu_5)/3 = 0$
Contrast 3 (C3)	$\mu_3 - \mu_4 = 0$
Contrast 4 (C4)	$(\mu_3 + \mu_4)/2 - \mu_5 = 0$

all tests are conducted at significance level α, the overall significance level is greater than α. Suppose that the four *A* or *B* tests given above are independent of one another. If we let α = 0.05 for each test, the probability of avoiding a Type I error on each test is 1 – 0.05 = 0.95. By the multiplication theory for independent events, the probability of avoiding a Type I error on all four tests is therefore
$(0.95)\,(0.95)\,(0.95)\,(0.95) = (0.95)^4 \approx 0.8145$, which means that the probability of making a Type I error on at least one of the tests is approximately 1 – 0.8145 = 0.1855. That is, our overall α is not 0.05, but about 0.18. In general, if K independent tests are conducted at level of significance α, the probability of at least one Type I error is $1 - (1 - \alpha)^K$. The assumption was made that the four tests in *A* or *B* above were independent. In the general case of J samples, the largest possible number of independent differences is J – 1. For three or more samples, the number of possible pairs J(J – 1)/2 is always greater than J – 1, so if one tests every difference, some tests will necessarily be independent. When all K tests are not independent, the overall probability of a Type I error may be impossible to calculate, but if all tests are conducted at significance level α, the overall probability can be as great as Kα, assuming, of course, that Kα does not exceed 1. One way to resolve the problem is to test every difference at a significance level of α/K. This ensures that the overall probability of a Type I error is no greater than α, but the dependency among tests makes multiple results difficult to interpret. A strategy that circumvents these difficulties was developed by Fisher in the 1920's and hinges on the reasoning that differences among J population means $\mu_1, \mu_2, \ldots, \mu_J$ should be reflected in variability among the means of samples drawn from these populations $\bar{x}_1, \bar{x}_2, \ldots, \bar{x}_J$.

(ii) A choice of 4 mutually orthogonal linear contrasts for the 5 means $\boldsymbol{\mu}_1$, $\boldsymbol{\mu}_2$, $\boldsymbol{\mu}_3$, $\boldsymbol{\mu}_4$, $\boldsymbol{\mu}_5$ is displayed in Table 4, with corresponding null hypotheses in Table 4a. In Table 5 a comparison is made between results obtained

using the method of mutually orthogonal linear contrasts[4] and the method of Harris[2].

Discussion

A number of interesting and unexpected results emerged from the respective statistical hypotheses tests. In the context of this paper, the term "significant difference" means the drug had an effect, while the term "no significant difference" means the drug had no effect.

For (i) in the example, the comparison of $\boldsymbol{\mu}_1$ and $\boldsymbol{\mu}_2$ shows a significant difference at a 5% and at a 1% level of significance. This result is extremely interesting. What could have caused this difference since Mydriacyl had not yet been instilled into the subject's right eye? Influencing factors could be the surrounding environment in which the experiment was conducted (air-conditioning of the room, lighting), changes in the tear layer, eye-lid movement during blinking, concentration, attention, other non-visual sensory inputs and motivation. Various factors such as disease processes, pharmacological agents and inner body changes (such as enzymatic or hormonal processes) can also affect refractive state.

At a 5% and at a 1% level of significance, hypotheses tests *A2, A3, A4* and *B4* show a significant difference. Hypothesis test *B3* shows a significant difference at a 5% level of significance, but no significant difference at a 1% level of significance. Test *B2* shows no significant difference at both levels of significance. This is a rather strange result.

For (ii) in the example, contrasts C1, C2 and C4 show a significant difference at a 5% level of significance, but at a 1% level of significance (a tighter restriction), only in cases C1 and C2 are there significant differences. Contrast C1 confirms the results obtained for *A1* and *B1*. Contrast C2 confirms the results obtained by Lemmer[5]. Contrast C4 shows no significant difference at a 1% level of significance, while contrast C3 shows no significant difference at both levels of significance. Analyse this case of non-effect of the drug for the contrast C3 more carefully. Many external factors could have affected these results. Maybe the subject was fatigued and thirsty, or there was a drop in blood pressure or glucose level at this point in time during the experiment. The result for contrast C4 at a 1% level of significance is acceptable since the average of the mean refractive state measured at 15 minutes and 30 minutes respectively post instillation is being compared with the mean refractive state 60 minutes post instillation when we expect the drug to have virtually worn off.

For the comparison of multivariate means using the method of Harris[2] in (ii), there is no significant difference at both levels of significance between $\boldsymbol{\mu}_3$ and $\boldsymbol{\mu}_4$, thus confirming the result obtained using contrast C3. No significant difference is observed at both levels of significance between the average of $\boldsymbol{\mu}_3$ and $\boldsymbol{\mu}_4$ with $\boldsymbol{\mu}_5$. However with contrast C4 there is no significant difference only at a 1% level of significance. When comparing the average of $\boldsymbol{\mu}_1$ and $\boldsymbol{\mu}_2$ with the average of $\boldsymbol{\mu}_3$, $\boldsymbol{\mu}_4$ and $\boldsymbol{\mu}_5$, the results using Harris[2] compare well with those of contrast C2 at both levels of significance.

Looking carefully at all of the above results, one may ask whether Mydriacyl is in fact an effective cycloplegic for paralysis of the ciliary muscle for the duration of this experiment.

It is interesting to note that, when comparing

Table 5: Test statistics for hypotheses tests on mean refractive state. The respective null hypotheses (H_0) are those presented Table 4a. Critical values are: (for mutually orthogonal linear contrasts: $F_{0.05,\ 3,\ 143} = 2.673$ and $F_{0.01,\ 3,\ 143} = 3.918$) and (for comparison of multivariate means: $F_{0.05,\ 3,\ 56} = 2.776$, and $F_{0.01,\ 3,\ 56} = 4.166$).

Test statistic using equation 1, with decision on H_0 denoted by * and **	**Test statistic using equation 22^2, with decision on H_0 denoted by * and ****
9.0939 * and **	6.9286 * and **
14.1924 * and **	5.7275 * and **
1.6363	2.0664
3.0058 *	1.9704

* Reject H_0 at 5% level of significance.

** Reject H_0 at 1% level of significance.

No asterisks: Retain H_0 at the appropriate levels of significance.

the results obtained using mutually orthogonal linear contrasts with those obtained using Harris[2], the value of the computed test statistic is often larger in the former case, thus making the chance of rejecting the null hypothesis more probable.

It is important to transform the comparisons of interest into contrasts. It is also important that the researcher understand the nature of contrasts when he or she interprets the contrast coefficients. Once comparisons are transformed into contrasts, the remaining procedure for testing their effects is relatively straightforward.

The introduction and application of mutually orthogonal linear contrasts to sample data in optometric research has been achieved in a clear, easy and understandable way. This very important concept should always be applied by the optometrist involved in such research. The specific set of contrasts that the researcher tests, depends on the questions that need to be addressed by the study. It is important to note that univariate contrasts should only be interpreted if the corresponding multivariate contrast is significant.

Conclusions

A sample size of 30 measurements per sample is possibly too small. A larger sample of 30-year-old female subjects with a variety of spectacle prescriptions should be subjected to the experimental procedure to draw more concrete conclusions. Possibly a control group should also be included. Subjects can be their own controls as in Gillan's approach. In addition one should also perform the experimental procedure on an equivalent sample of 30-year-old males including a control group. The experimental trial should also be performed on a combined group of 30-year-old males and females with a control group.

A study using Mydriacyl with autorefraction at near rather than at distance would also be useful to obtain a clearer idea of its cycloplegic efficacy. The entire experiment could be repeated with different cycloplegics, and the results compared to make the study more comprehensive.

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