TITLE

Consideration of sex as a biological variable in diabetes research across twenty years

AUTHORS

Celena Cherian[1*], Hayley Reeves[1,2*], Duneesha De Silva[1,3&], Serena Tsao[1,4&], Katie E. Marshall[5], Elizabeth J. Rideout[1§]

AFFILIATIONS

[1] Department of Cellular and Physiological Sciences, Life Sciences Institute, The University of British Columbia, Vancouver, Canada.

[2] Present address: School of Molecular Biosciences, University of Glasgow, Glasgow, UK.

[3] Present address: Department of Orthopaedics, The University of British Columbia, Vancouver, Canada.

[4] Department of Anatomy and Cell Biology, Faculty of Medicine and Health Sciences, McGill University, Montréal, Canada.

[5] Department of Zoology, The University of British Columbia, Vancouver, Canada.

[*] These authors contributed equally to this work

[&] These authors contributed equally to this work

[§] Corresponding author

CORRESPONDING AUTHOR DETAILS

Elizabeth J. Rideout, Life Sciences Center, 2350 Health Sciences Mall (RM3308), Vancouver, BC, Canada V6T 1Z3. Email: elizabeth.rideout@ubc.ca

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ABSTRACT

Sex differences exist in the risk of developing both type 1 and type 2 diabetes, and in the risk of developing diabetes-associated complications. Sex differences in glucose homeostasis, islet and β cell biology, and peripheral insulin sensitivity have also been reported in multiple animals. To determine the degree to which biological sex has been addressed in published literature related to diabetes and insulin biology, we developed a scoring system to assess the inclusion of biological sex in papers related to these topics. We scored manuscripts published in *Diabetes*, published by the American Diabetes Association, as this journal focuses on diabetes and diabetes-related research. We scored papers published across three years within a 20-year period (1999, 2009, 2019), a timeframe that spans the introduction of funding agency and journal policies to improve the consideration of biological sex as a variable. Our analysis shows fewer than 15% of papers used sex-based analysis in even one figure across all study years, a trend that was reproduced across journal-defined categories of diabetes research (*e.g.*, islet studies, signal transduction). Single-sex studies accounted for approximately 40% of all manuscripts, of which >87% used male subjects only. While we observed a modest increase in the overall inclusion of sex as a biological variable during our study period, our results highlight significant opportunities to improve consideration of sex as a biological variable in diabetes research. In particular, we show that journal policies represent one way to promote better consideration of biological sex as a variable. In the long term, improved practices will reveal sex-specific mechanisms underlying diabetes risk and complications, generating insights to support the development of sex-informed prevention and treatment strategies.

52 **INTRODUCTION**

53 Diabetes mellitus is a disease that affects millions of individuals globally. According to the
54 International Diabetes Federation, approximately 537 million individuals (20-79 years)
55 worldwide live with diabetes and it has caused 6.7 million deaths[1]. There are also rare
56 monogenic forms of diabetes that affect many people[2]. While diverse factors influence the risk
57 of developing diabetes[3–9], there is growing recognition that biological sex impacts the risk of
58 developing diabetes and diabetes-related complications[8–13]. In several populations, the risk of
59 developing type 2 diabetes (T2D) is approximately 40% higher in males than in
60 females[8,9,11,12,14,15]. In adults aged 15-40, males also have a 60% higher risk of developing
61 type 1 diabetes (T1D)[16]. This male bias in T1D is particularly notable given that females are at
62 a higher risk of nearly all other autoimmune-related diseases[17–19]. After diabetes onset,
63 females are at higher risk of diabetic heart disease, stroke mortality, depression, and anxiety,
64 whereas males show faster progression of nephropathy and require lower extremity
65 amputation more often[9,20–25].

66 Clues into factors that contribute to sex differences in the risk of developing diabetes
67 and diabetes-related complications include a male-female difference in peripheral insulin
68 sensitivity. In humans and many animal models[26–29], females typically show higher insulin
69 sensitivity[30–34]. Given the established links between reduced peripheral insulin sensitivity and
70 T2D[27,34–41], and between insulin sensitivity and diabetes-associated complications[9,21–25,42],
71 biological sex is an important variable to consider when studying insulin sensitivity across
72 physiological and pathological contexts. Sex differences also exist across many animals,
73 including humans, in the biology of insulin-producing β cells[30,43–47]. Male-female differences
74 have been reported in the number of pancreatic β cells[47], and studies in humans and rodents
75 report profound sex differences in β cell gene expression, function, and stress responses in

both normal and T2D contexts[30–34,48,49]. Female β cells also show higher glucose-stimulated insulin secretion under normal physiological conditions and in T2D[9,30–32], differences that cannot be solely attributed to sex differences in peripheral insulin sensitivity[30]. Because there are established links between β cell dysfunction in rodent models and humans in T2D[49–55], and emerging evidence suggests β cell dysfunction may also contribute to T1D[56,57], biological sex is an important variable to consider in studies on islets and β cells in multiple physiological and pathological contexts. Yet, the degree to which biological sex has been considered in the literature with respect to islet and β cells is incompletely known.

Broad-based text searches of diabetes articles, and detailed assessments of randomized controlled trials and human observational studies in top diabetes journals, suggest that biological sex is rarely considered as a variable in diabetes research[58–61]. Indeed, one study showed that only 6.5% of randomized controlled trials and human observational studies reported all outcomes separately according to biological sex[58]. While these studies were critical in highlighting the overall poor consideration of both sexes in diabetes research, several questions remain. For example, the prior focus on randomized controlled trials and observational studies means that we lack detailed information on whether biological sex is included in studies that focus on cell, tissue, and animal models of diabetes. We similarly lack information on whether there are differences in practices related to biological sex between different diabetes subject areas (*e.g.* islet biology, transplantation), and whether funding agency policies mandating the inclusion of both sexes[59,62–65] lead to better consideration of biological sex in diabetes research. Answering these questions will highlight opportunities for improvement in research design, methods, and analysis across all areas of diabetes research, and establish whether policies introduced by major funding

agencies, journals, and governments have produced sufficient change in research practices[62–66].

We performed a detailed analysis of all manuscripts published in the journal *Diabetes*, published by the American Diabetes Association, in 1999, 2009, and 2019 (819 publications) (Fig. 1A). Studying the inclusion of sex as a biological variable in one journal rather than between journals provides advantages due to uniform journal policies and similar manuscript quality. Because *Diabetes* publishes papers in multiple subject areas, and in both clinical and biomedical disciplines, we captured and compared differences in common practices across diverse fields related to diabetes mechanisms and risk. Overall, our findings suggest that fewer than 15% of studies published in *Diabetes* during our study period analyzed data using biological sex as a variable. Approximately 34% of studies included both sexes in data collection, but did not analyze their data using biological sex as a variable, and 40% of studies included only a single sex. While we observed a significant increase in the inclusion of both sexes in diabetes research over time, we observed a smaller increase in the number of studies that used sex-based analysis over time. These trends were broadly similar across many subfields of diabetes research, and funding status had no significant effect on overall consideration of biological sex as a variable in diabetes research. Together, our data suggests progress has been made in diabetes-related research with respect to the inclusion of both sexes and uptake of sex-based analysis; however, significant opportunities for improvement remain. This is especially true for single-sex studies, the vast majority of which used male and not female animals. Greater knowledge of how biological sex affects diabetes mechanisms and complications will ultimately support the development of sex-informed prevention and treatment strategies to improve equity in health outcomes[61,66].

RESULTS

Assessing the inclusion of biological sex as a variable in diabetes research over time

The start of the twenty-year period we considered in our study corresponded with the introduction of major policy initiatives aimed at improving the consideration of biological sex as a variable in Canada, Europe, and the United States[62–66]. To determine whether the inclusion of biological sex as a variable in diabetes research changed over time, we monitored the average score of the papers in our study in 1999, 2009, and 2019. The average score of papers published in 1999 was 1.47 ± 0.07, whereas the average scores of manuscripts published in 2009 and 2019 were 1.91 ± 0.073 and 2.28 ± 0.1, respectively (Fig. 1B). While this represents a significant increase in average score between 1999 and 2019 (year effect $p<0.001$; ANOVA in a generalized linear model with Poisson distribution), an increase in average score of only 0.8 over 20 years indicates that significant room for improvement remains in the inclusion of sex as a biological variable in diabetes research.

To describe the dynamics underlying the change in average score over time in more detail, we examined the proportion of studies classified into each of our six categories. The proportion of studies assigned a score of 0 significantly declined over time, from 27% in 1999, to 19% in 2009, and 7.3% in 2019 (year effect $p<0.001$; ANOVA in a generalized linear model with binomial distribution) (Fig. 1C). In contrast, there was a significant increase in the proportion of papers over time assigned a score of 3, 4, or 6 (year effect $p=0.002$, $p=0.004$, $p=0.029$ respectively; ANOVA in a generalized linear model with binomial distribution) (Fig. 1C). Papers assigned a score of 3 comprised 25.5% of papers in 1999, 36.8% in 2009, and 37.6% in 2019 (Fig. 1C). Papers assigned a score of 4 made up 4.2% of papers in 1999, 6% papers in 2009, and 10.6% papers in 2019, whereas papers assigned a score of 6 represented only 0.91% of studies in 1999, 1.6% in 2009, and 3.9% in 2019 (Fig. 1C).

148 No significant change was observed in the proportion of papers scored as a 1, 2, or 5
149 between 1999 and 2019 (year effect p=0.618, p=0.519, p=0.991 respectively; ANOVA in a
150 generalized linear model with binomial distribution) (Fig. 1C). Based on this data, the
151 categories with the most pronounced changes between 1999 and 2019 were 0 (decrease)
152 and 3 (increase) (Fig. 1C). This suggests the higher average score in 2019 may be partly
153 attributed to a drop in studies that did not discuss biological sex at all and an increase in
154 mixed-sex studies with no sex-based analysis. Increased inclusion of both sexes represents a
155 promising step toward better consideration of biological sex as a variable in diabetes
156 research; however, important next steps for improvement include greater use of sex-based
157 analysis.
158
159 **Male animals are used more often in single-sex studies**
160 Previous studies on the inclusion of biological sex as a variable in biomedical research reveal
161 that male animals are used more frequently than female animals[59,60,67]. For example, a study
162 from 2010 analyzed almost 2,000 animal studies across 10 biological fields in several journals
163 and found a male bias in eight fields, with the most pronounced bias in neuroscience,
164 pharmacology, and physiology[60]. To determine whether one sex was used more often in
165 diabetes research, for all papers assigned a score of 1 or 2 (single-sex studies) we recorded
166 the sex that was used. Studies assigned a score of 1-2 represented 42% of studies in 1999,
167 39.7% of studies in 2009, and 40% of studies in 2019 (Fig. 1D), suggesting single-sex studies
168 represented a large proportion of total studies published during this period. In 1999, 71.7% of
169 single-sex studies used only male animals (Fig. 1D). In 2009 and 2019, 82.2% and 87.3% of
170 single-sex studies used only male animals, respectively (Fig. 1D). When we examined the
171 number of male-only studies over time, we observed a significant increase between 1999 and
172 2019 (year effect p=0.023; ANOVA in a generalized linear model with binomial distribution)

173 (Fig. 1D). This suggests that in diabetes research, as in other fields of biomedical
174 science[59,60,67], male animals are used more frequently than female animals.
175
176 **Evaluating the inclusion of biological sex as a variable in diabetes research across**
177 **clinical and biomedical disciplines**
178 Diabetes research includes both clinical and biomedical disciplines. Given that policies
179 governing the inclusion of biological sex as a variable were introduced at an earlier date for
180 clinical research compared with biomedical research[59,60,68,69], we compared the average score
181 assigned to studies in each of these disciplines in 1999, 2009, and 2019. Across all three
182 study years, the average score for papers in the clinical discipline (2.78 ± 0.08) was
183 significantly higher than the average score for papers in the biomedical discipline (1.48 ±
184 0.05) (discipline effect $p<0.001$; ANOVA in a generalized linear model with Poisson
185 distribution) (Fig. 2A). We observed a significant increase in the average scores across both
186 biomedical and clinical disciplines between 1999 and 2019 (year: discipline interaction
187 $p<0.0017$; ANOVA in a generalized linear model with Poisson distribution) (Fig. 2A), with a
188 trend toward a larger increase over time in the biomedical discipline.
189 To identify factors that contribute to the score difference between the biomedical and
190 clinical disciplines, we examined the probability that a study in each category would be
191 assigned a specific score (Fig. 2B,C). While there was a significant decrease in the proportion
192 of both biomedical and clinical studies assigned a score of 0 over time (year effect $p<0.001$;
193 ANOVA in a generalized linear model with binomial distribution), the probability that a
194 biomedical study would be assigned a score of 0 was higher than the probability of a clinical
195 study being assigned this score across all study years (discipline effect $p<0.001$; generalized
196 linear model with binomial distribution) (Fig. 2B,C). The probability of a biomedical study

197 receiving a score of 1 was also significantly higher than the probability of a clinical study being
198 assigned this score in all three study years (discipline effect $p<0.001$; generalized linear
199 model with binomial distribution) (Fig. 2B).

200 In the clinical discipline, the score that was assigned to the highest proportion of
201 studies across all years was 3 (61.7% of studies; Fig. 2C). Indeed, the probability of a clinical
202 study being assigned a score of 3, 4 or 5 was significantly higher than the probability of a
203 biomedical study being assigned these scores in all study years (discipline effect $p<0.001$,
204 $p=0.002$ and $p=0.03$, respectively; generalized linear model with binomial distribution) (Fig.
205 2B,C). While our data indicate that more authors indicated the sex of the model system they
206 used in 2019 than in 1999, significant room for improvement remains. For example, in 2019
207 the majority of biomedical studies still used only a single sex (~50%) and most failed to use
208 sex-based analysis (86.6%). In the clinical discipline, fewer than 15% of published studies
209 used sex-based analysis during our study period.

210

211 **Comparing the inclusion of biological sex as a variable between diabetes subject areas**

212 Beyond the diverse approaches to diabetes research between clinical and biomedical
213 disciplines, diabetes research spans a broad range of topics, from genetic and genomic
214 approaches, to immunology, to pharmacology. We therefore wanted to compare the inclusion
215 of biological sex as a variable across different diabetes subject areas. From 2009 onwards, all
216 studies published in *Diabetes* correspond to one of 10 primary topics/subjects (Table 2). Note
217 that not all areas maintained identical labels in 2009 and 2019. For example, the topic
218 'Genetics' in 2009 was compared to 'Genetics/Genomes/Proteomics/Metabolomics' in 2019
219 (hereafter called Genetics/Omics). Similarly, the 'New Methodologies and Databases' was
220 compared to 'Technological Advances' in 2019 (hereafter called New Methodology and
221 Technology). To ensure adequate sample sizes within each topic, we excluded subject areas

222 with fewer than ten papers in either 2009 or 2019 (Signal Transduction, New Methodology
223 and Technology, Pharmacology & Therapeutics).
224 In 2009, the average score was highest in the Genetics/Omics and Obesity subject
225 areas (2.5 ± 0.17 and 2.62 ± 0.39, respectively), and the lowest in Immunology and
226 Transplantation and Islet topics (1.62 ± 0.27 and 1.35 ± 0.23, respectively; Fig. 3A). In 2019,
227 the average scores were highest in the Genetics/Omics, Obesity, and Immunology and
228 Transplantation topics (3.04 ± 0.20, 2.67 ± 0.34, and 2.72 ± 0.38, respectively) (Fig. 3A). In
229 both 2009 and 2019, the higher score for studies under the Genetics/Omics subject area may
230 be attributed to the higher probability of a study in that subject area being assigned a score of
231 3 compared with other subject areas (Fig. 3B-H). Despite the different average scores
232 between subject areas, our model suggests that all subject areas showed a similar change in
233 average score over time (year:subject interaction p=0.341; ANOVA in a generalized linear
234 model with Poisson distribution). Thus, our analysis did not reveal widespread differences in
235 practices related to the inclusion of biological sex as a variable between different diabetes
236 subject areas.
237
238 **Evaluating the effect of funding source on the inclusion of biological sex as a variable**
239 **in diabetes research**
240 Funding agencies across many countries have introduced policies aimed at increasing the
241 inclusion of biological sex as a variable in research[59,62–66]. The US National Institutes of
242 Health (NIH) represents a key source of funds for health research, with significant
243 investments in diabetes research[63]. The NIH was among the first funding agencies worldwide
244 to introduce policies related to the inclusion of both sexes in both clinical and biomedical
245 research[59,62,64–66]. To evaluate whether NIH funding affected the inclusion of biological sex as

246 a variable in diabetes research, we compared the average score assigned to studies by
247 authors that declared NIH funding with scores of studies by authors that did not declare NIH
248 funding (Fig. 4A). We found that the average score of NIH-funded studies was not
249 significantly higher than studies that declared funding from other sources (funding effect
250 p=0.067; year:funding interaction p=0.974; ANOVA in a generalized linear model with Poisson
251 distribution) (Fig. 4A). We next compared the proportion of studies within each score category
252 between NIH-funded studies and non-NIH-funded studies (Fig 4B,C). For the majority of
253 score categories (0-5), NIH funding had no significant effect on the proportion of studies
254 assigned a specific score (funding effect p=0.942, p=0.236, p=0.777, p=0.589, p=0.771,
255 p=0.222, respectively; ANOVA in a generalized linear model with binomial distribution) (Fig.
256 4B,C). In contrast, NIH-funded studies showed a significantly higher proportion of studies
257 assigned a score of 6 across all study years compared with non-NIH-funded studies (funding
258 effect p<0.001; funding:year interaction p=0.049; ANOVA in a generalized linear model with
259 binomial distribution) (Fig. 4B,C). This data indicates that NIH funding was not associated with
260 overall shifts in practices related to the consideration of biological sex as a variable in
261 diabetes research. Indeed, despite the fact that policies related to the inclusion of biological
262 sex as a variable in clinical research[16] were introduced earlier than similar policies related to
263 biomedical research[62,64,65], there was no significant interaction between year, funding source
264 and type of study when we asked whether NIH funding affected the average scores assigned
265 to clinical and biomedical studies (Fig. 4D). This reinforces our conclusion that NIH funding
266 was not associated with increased consideration of biological sex as a variable in diabetes
267 research.
268
269 **Evaluating the effect of journal policies on inclusion of sex as a biological variable**

270 In 2019, a new policy was introduced at *Diabetes* mandating that authors of studies that use
271 human islets provide detailed information on donor characteristics[70–72]. The goal of including
272 this detailed donor information was to enhance transparency and to enable better
273 comparisons among data generated using human islets. One of the required donor
274 characteristics included in the Human Islet checklist is biological sex[72]. Because disclosing
275 the sex of experimental models represents one way that authors can improve consideration of
276 biological sex in their research, we assessed the effect of this policy change on the scores
277 assigned to papers in the 'Islet studies' subject area before and after the policy came into
278 effect in April 2019. We scored all manuscripts published in this subject area in 2018 (36
279 papers) and 2020 (32 papers), as this was the topic most likely to be affected by a policy
280 regarding human islet documentation. We did not score papers published in 2019 as these
281 papers were likely submitted and/or reviewed prior to the policy change.

282 We found the average score of papers in the Islet subject area in 2018 was 1.66 ±
283 0.64, whereas the average score of studies in this area was 2.5 ± 0.19 in 2020 (Fig. 5A). This
284 represented an increase of 0.8 in the average score of studies in the Islet subject area over
285 only a two-year period (Fig. 5A). While this increase was not statistically significant
286 (year:subject interaction p=0.248; ANOVA in a generalized linear model with Poisson
287 distribution) it is worth noting that the magnitude of this increase is equivalent to the increased
288 we observed in all studies across our twenty-year study period. Potential explanations for the
289 increase in average score for studies in the Islet subject area include a trend toward more
290 studies assigned a score of 4-6, and a trend toward fewer studies assigned a score of 0-1
291 (Fig. 5B). To determine whether this increase was reproduced in another subject area less
292 likely to be affected by the policy change regarding human islets, we repeated our analysis of
293 manuscripts published in 2018 and 2020 in the 'Metabolism' subject area. The average score

294 for studies in the 'Metabolism' subject area increased by 0.03 over the span of the policy
295 change and was not significantly different in 2018 compared with 2020 (year:subject
296 interaction p=0.248; ANOVA in a generalized linear model with Poisson distribution) (Fig. 5A).
297 Taken together our analysis suggests that the editorial policy change at *Diabetes* may have
298 contributed to a significant improvement in the consideration of biological sex as a variable in
299 diabetes research.
300

301 **DISCUSSION**

302 The overall goal of our study was to assess the degree to which biological sex was included
303 as a variable in diabetes research across a twenty-year period. We chose to assess studies in
304 a journal that is dedicated to publishing high-quality studies in the area of diabetes research,
305 and across a timeframe that captures widespread changes to journal and funding agency
306 policies regarding sex- and gender-based analysis[62,64,65,70–72]. While our analysis revealed
307 significant increases in the inclusion of biological sex as a variable over time, in alignment
308 with the reported increase in the word "sex" in text-based searches of a similar body of
309 literature[58], our detailed evaluation of methods demonstrates that many studies do not
310 adequately address biological sex as a variable in diabetes research. Given that biological
311 sex affects diabetes risk[1,9,16,56], treatment efficacy[3,8,71], and risk of developing diabetes
312 complications[9,20,21,25,42], this knowledge gap must be closed in order to develop sex-informed
313 prevention and treatment strategies for people living with diabetes.

314 One major barrier to the full consideration of biological sex as a variable in diabetes
315 research was related to inclusion. In all three study years across a twenty-year period,
316 between 46 and 69% of studies did not include both sexes (score 0-2). This low inclusion of
317 both sexes was particularly notable in biomedical studies (between 59% and 81% of studies
318 across the 3 years were assigned a score of 0-2) and in diabetes subject areas that contained

319 a large proportion of biomedical studies (*e.g.,* islets and metabolism). A lack of inclusion of
320 both sexes has been described in studies of literature related to neuroscience, psychiatry and
321 psychology, pharmacology, and physiology[60,67,68,73,74], and our data aligns with findings from
322 others[58,68,69,73,75,76] suggesting similar trends exist in diabetes research. Indeed, the inclusion
323 of both sexes did not show a meaningful increase over a twenty-year period in many subject
324 areas of diabetes research. Addressing this overall lack of inclusion is an important task going
325 forward for the diabetes community as funding agencies and journals worldwide aim to
326 improve transparency and reproducibility in research[59,66]. Because our study showed the
327 majority of single-sex studies use only male subjects, the major change that is needed in
328 biomedical studies is the inclusion of female subjects. Indeed, our analysis suggests that the
329 gap in knowledge of female cells and animals will not be closed if current trends in single-sex
330 studies continue. Given that female subjects are protected from dysregulation of glucose
331 homeostasis[27,30–32,34,37,38], but are at a higher risk of developing complications from
332 diabetes[9,21,42], mechanistic insights gained from studying females will provide valuable
333 insights into diabetes prevention and treatment.

334 While a lack of inclusion of both sexes is an important barrier to consideration of
335 biological sex as a variable in diabetes research, it is important to note that single-sex studies
336 are acceptable in some cases. For this reason, we assigned a higher score (2) to a small
337 number of manuscripts (n=34) that provided a reason for their use of a single-sex study
338 group. When we examined the reasons provided by the authors of manuscripts assigned a
339 score of 2 for their choice of using a single-sex study group, we found two broad categories of
340 justifications. In the first category, the reason was that the single-sex study group included
341 pregnant people or individuals living with polycystic ovary syndrome. Because these contexts
342 are not experienced by both sexes, it is acceptable to use a single-sex group in these studies.
343 Future studies will need to adjust our scoring system to reflect the fact that using single-sex

344 study groups with a strong justification considers biological sex properly, and should perhaps
345 score higher than mixed-sex studies.

346 The second category of studies assigned a score of 2 included manuscripts that
347 provided a variety of reasons for including only a single sex. Reasons given for using a single-
348 sex study group in this category included a lack of prior knowledge in one sex and fluctuating
349 female hormones (Supplemental file 1). While a lack of detailed knowledge of diabetes
350 mechanisms in both sexes is an unfortunate consequence of the fact that few studies properly
351 consider biological sex as a variable, it does not represent a strong justification for using a
352 single-sex study group. Similarly, several studies now show that female animals are not more
353 variable than males across multiple traits[77,78]. These are therefore not strong justifications for
354 the failure to include both sexes in a study.

355 Another major barrier to the full consideration of biological sex as a variable in diabetes
356 research was a lack of sex-based analysis. Studies assigned a score of 3 comprised between
357 17% and 28% of those published in the biomedical discipline and between 51% and 77% of
358 clinical studies between 1999 and 2019. In the biomedical discipline, the most common
359 reason studies were assigned a score of 3 was that they pooled samples (*e.g.*, islets)
360 collected from male and female experimental models prior to collecting measurements. This
361 suggests that in biomedical studies, the major changes that are needed to support sex-based
362 analysis are to 1) collect samples and data from male and female experimental models
363 separately, and 2) to use appropriate statistical methods to analyze the data from each sex
364 separately. In clinical studies, the main reason studies were assigned a score of 3 was that
365 biological sex was not treated as a variable. Instead, most studies in the clinical discipline
366 considered biological sex as a covariate. Given that biological sex can interact with treatment
367 to drive outcomes, including biological sex only as a covariate can mask sex-dependent
368 effects. Biological sex should therefore be included either as an interactor, or analyses should

369 be stratified by sex[74]. The main change that is needed in the clinical discipline is therefore to
370 adjust study analysis methods to fully consider biological sex as a variable. Because many
371 studies show sex differences in islet and β cell function[41,47,48,79], the genetic architecture of
372 disease[9,41,42,55], and metabolic differences[27,30,32,33,38], shifts in practice across the biomedical
373 and clinical disciplines, and in different diabetes subject areas, will allow researchers to
374 develop evidence-based models for these topics in each sex. These models will form the
375 basis for the development of sex-informed prevention and treatment efforts for individuals at
376 risk for, or living with, diabetes.

377 Despite the growing number of studies showing that biological sex is not given
378 adequate consideration in biomedical and clinical research[60,68,69,80], the most effective
379 strategies to promote better uptake of biological sex as a variable are not fully understood.
380 Our data, in alignment with similar studies[73,75,76], indicate that funding by agencies with
381 clearly-stated requirements related to the consideration of biological sex does not significantly
382 influence author practices (Fig. 4). One potential reason for this lack of influence on author
383 practices is that monitoring compliance with sex- and gender-related policies poses multiple
384 challenges. Due to a policy change during the course of our study, we were able to assess
385 how journal policies affect the consideration of biological sex as a variable. Our data suggests
386 that journal policies may represent an effective tool in promoting the uptake and integration of
387 biological sex as a variable in diabetes research. In the 'Islet studies' subject area, the
388 increase in average score of 0.8 between 2018 and 2020 was similar in magnitude to the 0.91
389 increase in average score across the biomedical discipline over a twenty-year period. This
390 suggests the implementation of robust journal policies related to integration of biological sex
391 as a variable will promote better practices in the future. Applying these policies will require a
392 culture shift across editors, reviewers, and authors, however, proper consideration of

393 biological sex as a variable will provide significant benefits in terms of data reproducibility,
394 transparency, and scientific rigor[69,73,75,80].

395 Based on our analysis, we suggest several practices authors can adopt to improve
396 consideration of biological sex as a variable in their research. First, we discuss changes that
397 can be made in the short term. For example, one way to immediately improve transparency
398 regarding integration of biological sex as a variable is to ensure the sex of experimental
399 subjects and materials are explicitly stated in the methods section and in figure legends. If
400 only a single sex is used, justification should be provided for the use of a single-sex
401 experimental group. Authors should also identify the use of a single sex as a limitation at
402 some point in the paper, and state that future studies will be needed to determine whether
403 findings made in one sex apply to the other. This is important even in studies that use cell
404 lines or tissues that are limited to one sex (*e.g.*, Chinese hamster ovary cell line), as it allows
405 readers to appreciate potential limitations of the mechanisms uncovered by the authors. If
406 both sexes are used, but not all phenotypes are recorded in each sex, the figure legends
407 should include this information to ensure the reader is aware of which sex was used to
408 generate specific data points.

409 It is also important to ensure data from each sex is collected and analyzed separately,
410 and not pooled. Pooling male and female cells or tissues during analysis risks overlooking
411 important effects if a sex-specific outcome does not achieve statistical significance within a
412 mixed-sex group. Similarly, potential interactions between biological sex and other variables
413 should be explicitly tested for, or the analysis should be stratified by sex, to allow for detection
414 of sex-specific effects. Treatment of biological sex as a covariate potentially masks those
415 effects. Because these suggestions are all related to changes in text, and how data are
416 displayed, described, and analyzed, they can be applied to studies that are either complete or
417 in progress. Longer-term changes include using male and female cell lines and animal

418 models, collecting and analyzing data from males and females separately, and applying
419 statistical methods to reveal how biological sex affects diverse phenotypes. The solid
420 foundation of knowledge that will emerge from these studies will help overcome the current
421 lack of information on male and female cells and animal models. It will also serve as a first
422 step in embracing diversity in sex chromosome complement and sex hormone levels, in line
423 with emerging recognition that biological sex is not a binary variable[13,61,69,74,76,80].
424 In conclusion, better knowledge of normal physiology in males and females will allow
425 the development of sex-informed prevention efforts, and better knowledge of pathophysiology
426 will support the discovery of more effective treatment options that reduce the burden of
427 complications. Broader consideration of biological sex as a variable in diabetes research will
428 therefore provide significant benefits to all individuals at risk for, and living with, diabetes.
429
430 **METHODS**
431
432 ***Article selection criteria*.** Our analysis included original research articles for which the full
433 text was available on the *Diabetes* online archive. We did not include reviews, editorials, or
434 commentaries in our analysis. We also excluded case studies, as reports on individuals
435 preclude the meaningful analysis of both sexes (Fig. 1A).
436
437 ***Scoring the inclusion of sex as a biological variable*.** Each paper was scored based on
438 the degree to which biological sex was integrated into study design and data analysis. A full
439 breakdown of the scoring system is included as Table 1. Briefly, a score of 0 was assigned to
440 studies in which the sex of animal models or participants was not stated at any point in the
441 text including the methods section. This includes studies that used cell lines with an obvious
442 sex (*e.g.*, Chinese hamster ovary cell line) in cases where the authors did not discuss the sex

443 of this cell line as a potential study limitation. A score of 1 was assigned to studies that used a
444 single sex (male or female) but did not provide any rationale for this choice. A score of 2 was
445 assigned to studies that used a single sex but provided an explicit reason for this decision. In
446 some cases the authors provided strong justifications for the use of a single sex, whereas
447 other authors provided less strong justifications for their choice. Strong justifications for the
448 use of a single sex included studies on pregnant subjects and individuals with polycystic
449 ovary syndrome. Less strong justifications included a lack of prior studies in one sex. A score
450 of 3 was given to mixed-sex studies. These studies included both sexes, but did not 1)
451 indicate the number of male and female subjects used and 2) analyze their data by sex.
452 Clinical studies that used biological sex as a covariate were also assigned a score of 3
453 because this type of analysis does not properly consider biological sex effects as a variable. A
454 score of 4 was given to studies that included biological sex as a variable in the analysis for as
455 little as one piece of data (*e.g.*, one panel of a figure reported male and female phenotypes
456 separately). A score of 5 was assigned to studies that included biological sex as a variable in
457 >50% of the data presented in the manuscript. A score of 6 was assigned for studies that
458 included both males and females across all experiments, and in which all data was analyzed
459 and reported by sex. The full data set is available in Supplemental Files 1 and 2.
460 ***Scoring reproducibility***. After the full data set was collected, a subset of articles was re-
461 scored by another person to assess inter-individual scoring variability. Of the 86 papers that
462 were selected at random for re-scoring, 96.5% (83/86) were assigned the same score by
463 different individuals. This indicates consistency between individuals in the application of our
464 scoring system.
465
466 ***Recording the sex of experimental subject.*** For papers with a score of '1' or '2' the sex of
467 the experimental subjects, as reported by the authors, was recorded in our spreadsheet.

468

469 ***Recording diabetes discipline and subfields.*** Each paper was classified as either 'clinical'
470 or 'biomedical' to allow a comparison between these two disciplines regarding the inclusion of
471 biological sex as a variable. Clinical studies included randomized controlled trials and studies
472 in which subjected human participants were subjected to treatment protocols. Observational
473 studies involving human subjects were also classed as clinical when material or recordings
474 beyond a single blood sample, superficial tissue, or DNA sample was taken for analysis. All
475 other papers, including those which used animal models or cell lines, were classed as
476 biomedical. For papers with both clinical and biomedical data, the classification was based on
477 the type of data from which the majority (>50%) of the results were generated. For papers
478 published in 2009 and 2019 the research subject area of each study was also recorded based
479 on the classification system used by *Diabetes* (*e.g.*, 'Islet biology', 'Obesity'; see Table 2).
480 Because papers published in 1999 were not categorized according to subject area, this
481 information was only collected for 2009 and 2019.

482

483 ***Funding status*.** We recorded the funding source for all papers in which this information was
484 reported by the authors.

485

486 ***Statistical analysis*.** All statistical analyses were performed using R[81]. For all analyses,
487 generalized linear models were used. For mean score changes, a Poisson error distribution
488 was assumed as scores are integers > -1. To calculate changes in proportions of scores, and
489 the percentage of male- and female-biased studies, the scores were converted to a binary
490 format and a binary (logistic) distribution was assumed. All analysis was started with a
491 maximal model and *p*-values from the resulting ANOVA table are reported. *p* values are
492 available in Supplemental File 3.

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718 **FIGURE LEGENDS**

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720 **Figure 1 – Assessing the inclusion of biological sex as a variable in *Diabetes***
721 **manuscripts published over time.** (A) Schematic representation of the workflow for
722 manuscript selection in our study. (B) Average score of studies published in 1999, 2009, and
723 2019. (*** $p<0.001$; generalized linear model with Poisson distribution; error bars indicate
724 SEM) (C) Proportion of studies assigned scores between 0-6 in 1999, 2009, and 2019.
725 Dashed lines represent a statistically insignificant change and solid lines represent a
726 statistically significant change (generalized linear model with binomial distribution). (D)
727 Proportion of single-sex studies that used male animals in 1999, 2009, and 2019 (** $p<0.01$;
728 generalized linear model with binomial distribution).

729

730 **Figure 2 – Evaluating the inclusion of biological sex as a variable in *Diabetes***
731 **manuscripts across clinical and biomedical disciplines.** (A) Average scores assigned to
732 studies published in biomedical and clinical disciplines in 1999, 2009, and 2019 (*** $p<0.001$;
733 generalized linear model with Poisson distribution; error bars indicate SEM). (B) Proportion of

734 biomedical studies assigned scores between 0-6 in 1999, 2009, and 2019. (C) Proportion of
735 clinical studies assigned scores between 0-6 in 1999, 2009, and 2019.
736
737 **Figure 3 – Comparing the inclusion of biological sex as a variable between *Diabetes***
738 **manuscripts published across diabetes subject areas.** (A) Average scores assigned to
739 studies published in different diabetes subject areas in 2009 and 2019. (ns not significant;
740 generalized linear model with Poisson distribution; error bars indicate SEM) (B) Proportion of
741 studies assigned scores between 0-6 in 2009 and 2019 in the 'Complications' subject area.
742 (C) Proportion of studies assigned scores between 0-6 in 2009 and 2019 in the
743 'Genetics/Omics' subject area. No studies in either year were assigned a score of 2 or 5. (D)
744 Proportion of studies assigned scores between 0-6 in 2009 and 2019 in the 'Immunology and
745 Transplantation' subject area. No studies in either year were assigned a score of 5 or 6. (E)
746 Proportion of studies assigned scores between 0-6 in 2009 and 2019 in the 'Islet' subject
747 area. No studies in either year were assigned a score of 5 or 6. (F) Proportion of studies
748 assigned scores between 0-6 in 2009 and 2019 in the Obesity subject area. (G) Proportion of
749 studies assigned scores between 0-6 in 2009 and 2019 in the Metabolism subject area. (H)
750 Proportion of studies assigned scores between 0-6 in 2009 and 2019 in the Pathophysiology
751 subject area. No studies in either year were assigned a score of 5 or 6.
752
753 **Figure 4 – Evaluating the effect of funding source on the inclusion of biological sex as**
754 **a variable in diabetes research** (A) Average scores assigned to studies that declared NIH
755 funding compared with studies that did not declare NIH funding in 1999, 2009, and 2019 (ns
756 not significant; generalized linear model with Poisson distribution; error bars indicate SEM).
757 (B) Proportion of NIH-funded studies assigned scores between 0-6 in 1999, 2009, and 2019.
758 (C) Proportion of non-NIH-funded studies assigned scores between 0-6 in 1999, 2009, and

759 2019. (D) Scores assigned to studies that declared NIH funding in the biomedical and clinical
760 disciplines in 1999, 2009, and 2019 (ns not significant; generalized linear model with Poisson
761 distribution; error bars indicate SEM).
762
763 **Figure 5 – Evaluating the effect of journal policies on inclusion of sex as a biological**
764 **variable in *Diabetes* manuscripts.** (A) Average scores assigned to studies in the 'Islet' and
765 'Metabolism' subject areas between 2018 and 2020 (before and after the 2019 policy change,
766 respectively) (** $p<0.01$; ns not significant; generalized linear model with Poisson distribution;
767 error bars indicate SEM). (B) Proportion of studies in the 'Islet' subject area assigned scores
768 between 0-6 in 2018 and 2020.
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778 **TABLE 1 – Scoring system to assess the consideration of biological sex as a variable**
779

Score	Meaning
0	No mention of sex
1	One sex used, without explicit reasoning
2	One sex used, with explicit reasoning; includes studies with strong and less strong justifications for this choice
3	Both sexes included; data not analyzed separately by sex or data not provided
4	Both sexes included; fewer than half of results analyzed by sex (<50%)
5	Both sexes included; more than half of results analyzed by sex (>50%)

6	Both sexes included; all data analyzed and reported by sex

782 **TABLE 2 – Number of papers in each diabetes subject area**

Diabetes subject area	Number of papers in 2009	Number of papers in 2019
Complications	41	37
Genetics / Omics (Genetics - 2009) (Genetics/Genomes/Proteomics/Metabolomics - 2019)	54	25
Immunology and Transplantation	24	11
Islet studies	37	25
Obesity studies	21	18
Metabolism	81	37
Pathophysiology	27	12
New Methodology / Technology (New Methodologies and Databases - 2009) (Technological Advances - 2019)	3	2
Pharmacology	8	9
Signal Transduction	16	2

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786

FIGURE 1

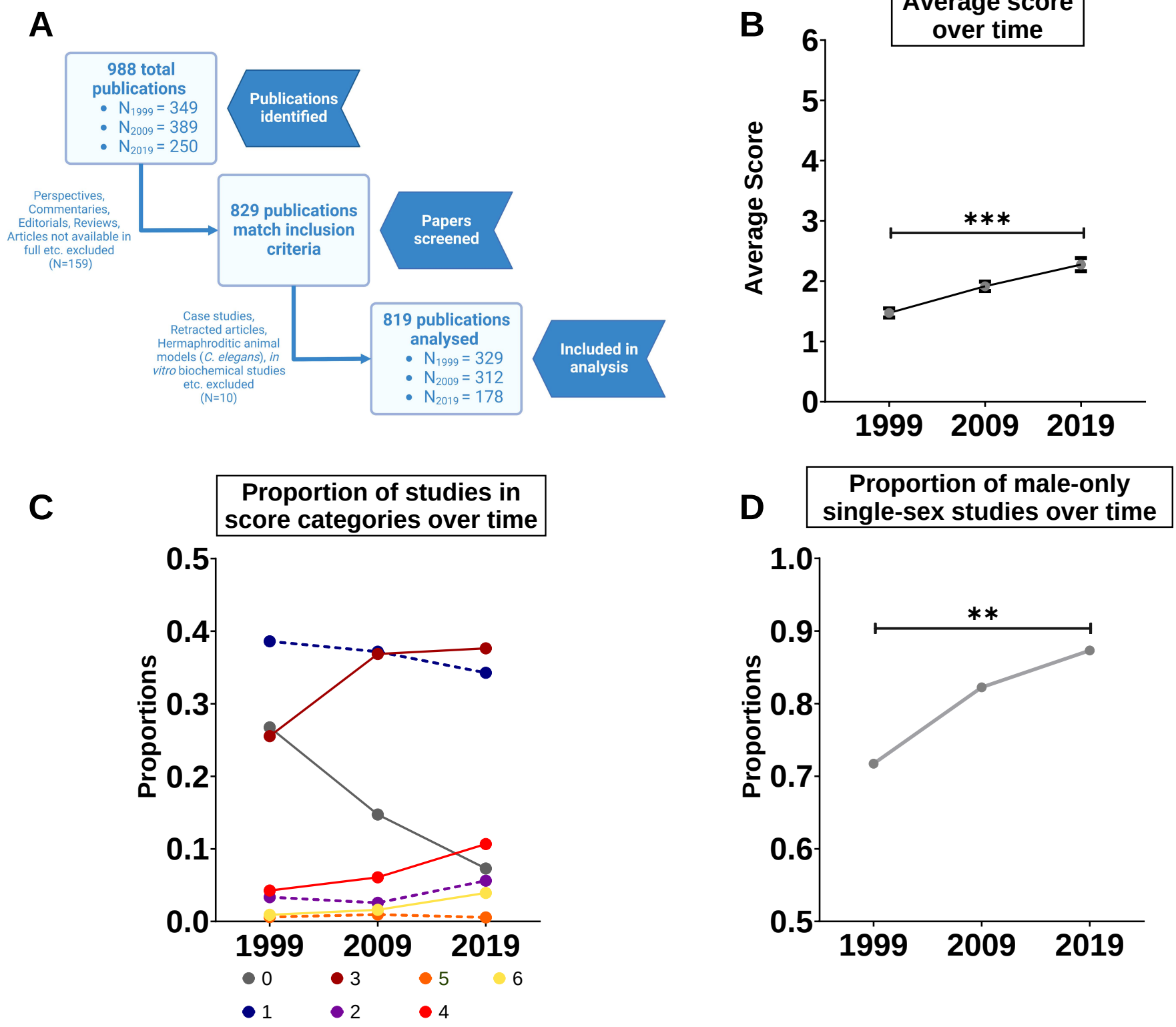

A
988 total publications
• N1999 = 349
• N2009 = 389
• N2019 = 250
Publications identified
Perspectives, Commentaries, Editorials, Reviews, Articles not available in full etc. excluded (N=159)
829 publications match inclusion criteria
Papers screened
Case studies, Retracted articles, Hermaphroditic animal models (C. elegans), in vitro biochemical studies etc. excluded (N=10)
819 publications analysed
• N1999 = 329
• N2009 = 312
• N2019 = 178
Included in analysis
B
Average score over time
Average Score
0
1
2
3
4
5
6
***
1999
2009
2019
C
Proportion of studies in score categories over time
Proportions
0.0
0.1
0.2
0.3
0.4
0.5
1999
2009
2019
0
1
2
3
4
5
6
D
Proportion of male-only single-sex studies over time
Proportions
0.5
0.6
0.7
0.8
0.9
1.0
**
1999
2009
2019

FIGURE 2

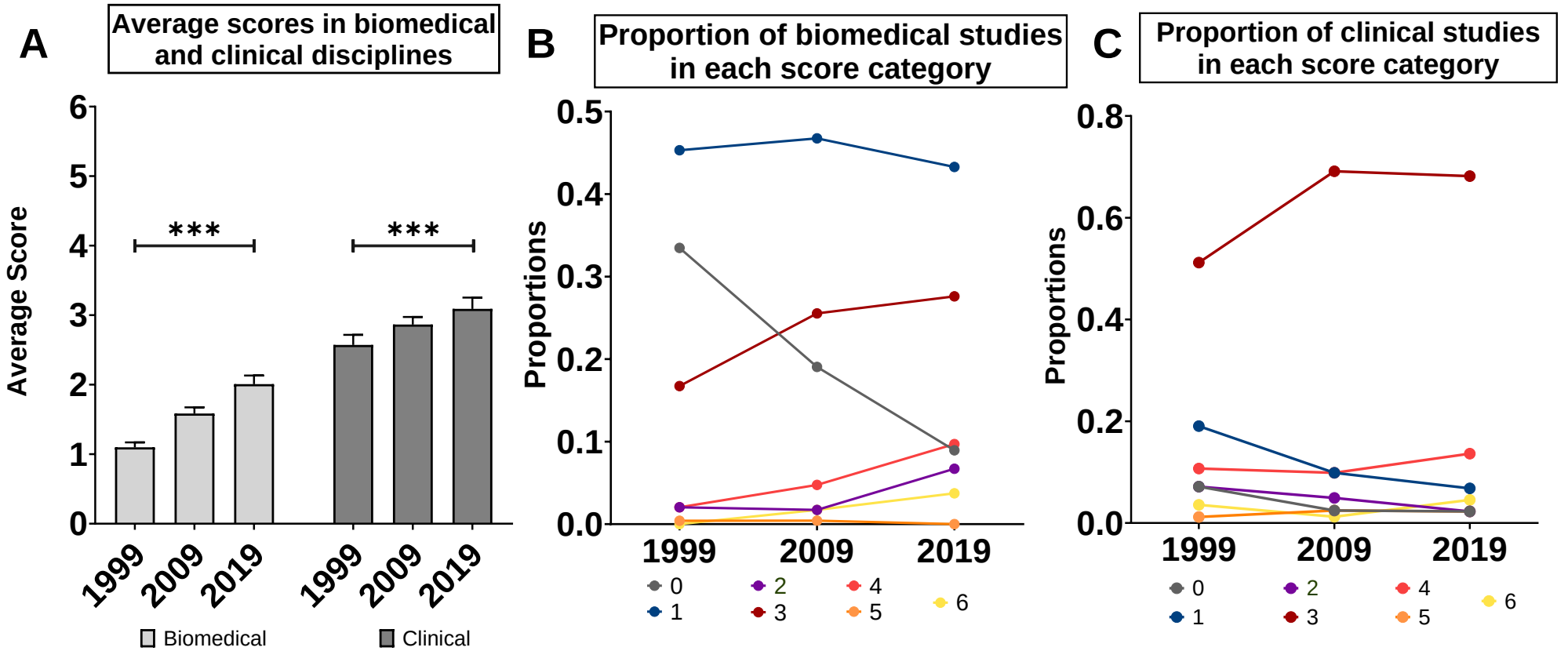

A
Average scores in biomedical and clinical disciplines
Average Score
6
5
4
3
2
1
0
***
***
1999
2009
2019
1999
2009
2019
Biomedical
Clinical
B
Proportion of biomedical studies in each score category
Proportions
0.5
0.4
0.3
0.2
0.1
0.0
1999
2009
2019
0
1
2
3
4
5
6
C
Proportion of clinical studies in each score category
Proportions
0.8
0.6
0.4
0.2
0.0
1999
2009
2019
0
1
2
3
4
5
6

FIGURE 3

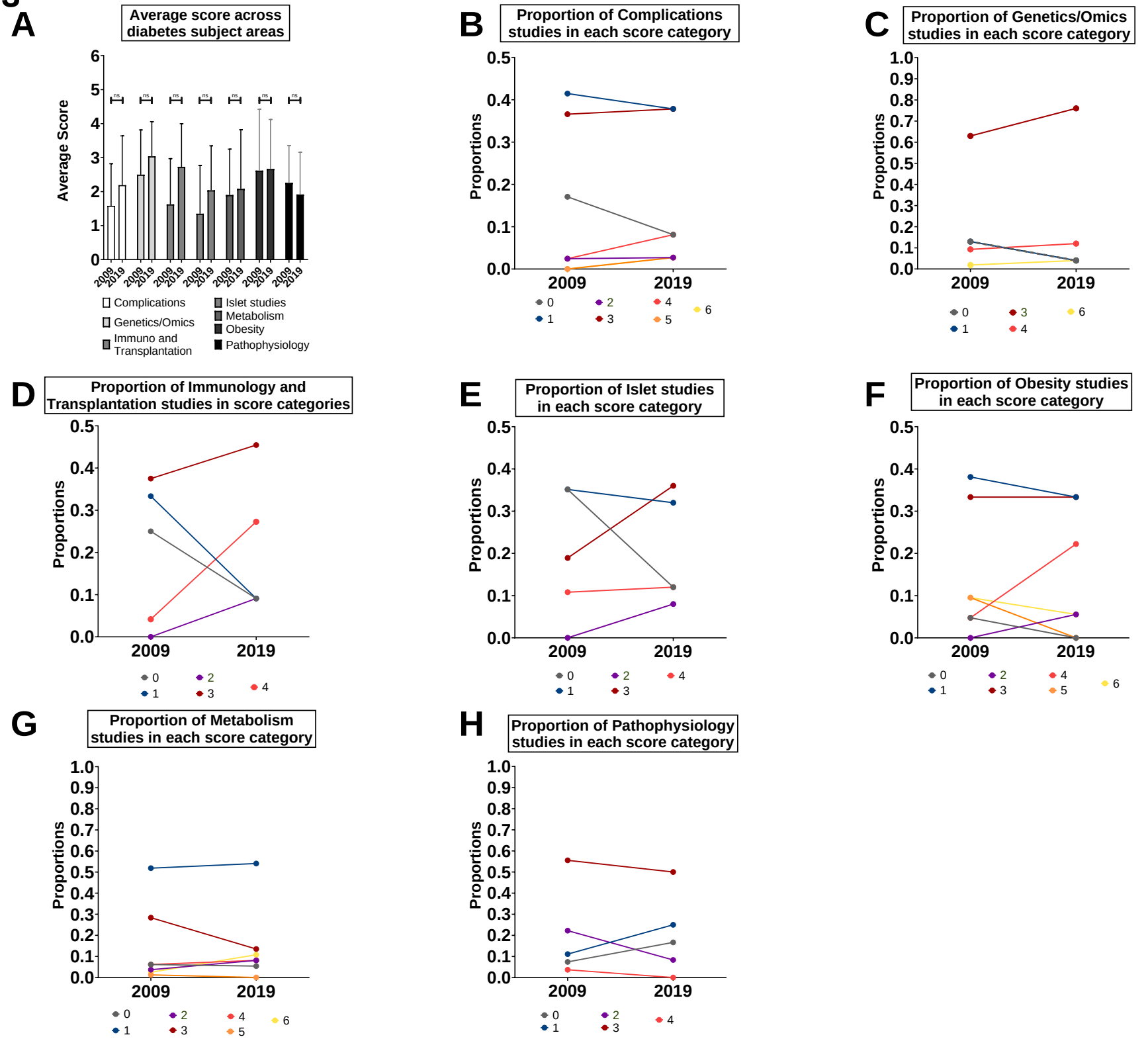

A
Average score across diabetes subject areas
Average Score
ns
2009
2019
Complications
Genetics/Omics
Immuno and Transplantation
Islet studies
Metabolism
Obesity
Pathophysiology
B
Proportion of Complications studies in each score category
Proportions
2009
2019
0
1
2
3
4
5
6
C
Proportion of Genetics/Omics studies in each score category
Proportions
2009
2019
0
1
3
4
6
D
Proportion of Immunology and Transplantation studies in score categories
Proportions
2009
2019
0
1
2
3
4
E
Proportion of Islet studies in each score category
Proportions
2009
2019
0
1
2
3
4
F
Proportion of Obesity studies in each score category
Proportions
2009
2019
0
1
2
3
4
5
6
G
Proportion of Metabolism studies in each score category
Proportions
2009
2019
0
1
2
3
4
5
6
H
Proportion of Pathophysiology studies in each score category
Proportions
2009
2019
0
1
2
3
4

FIGURE 4

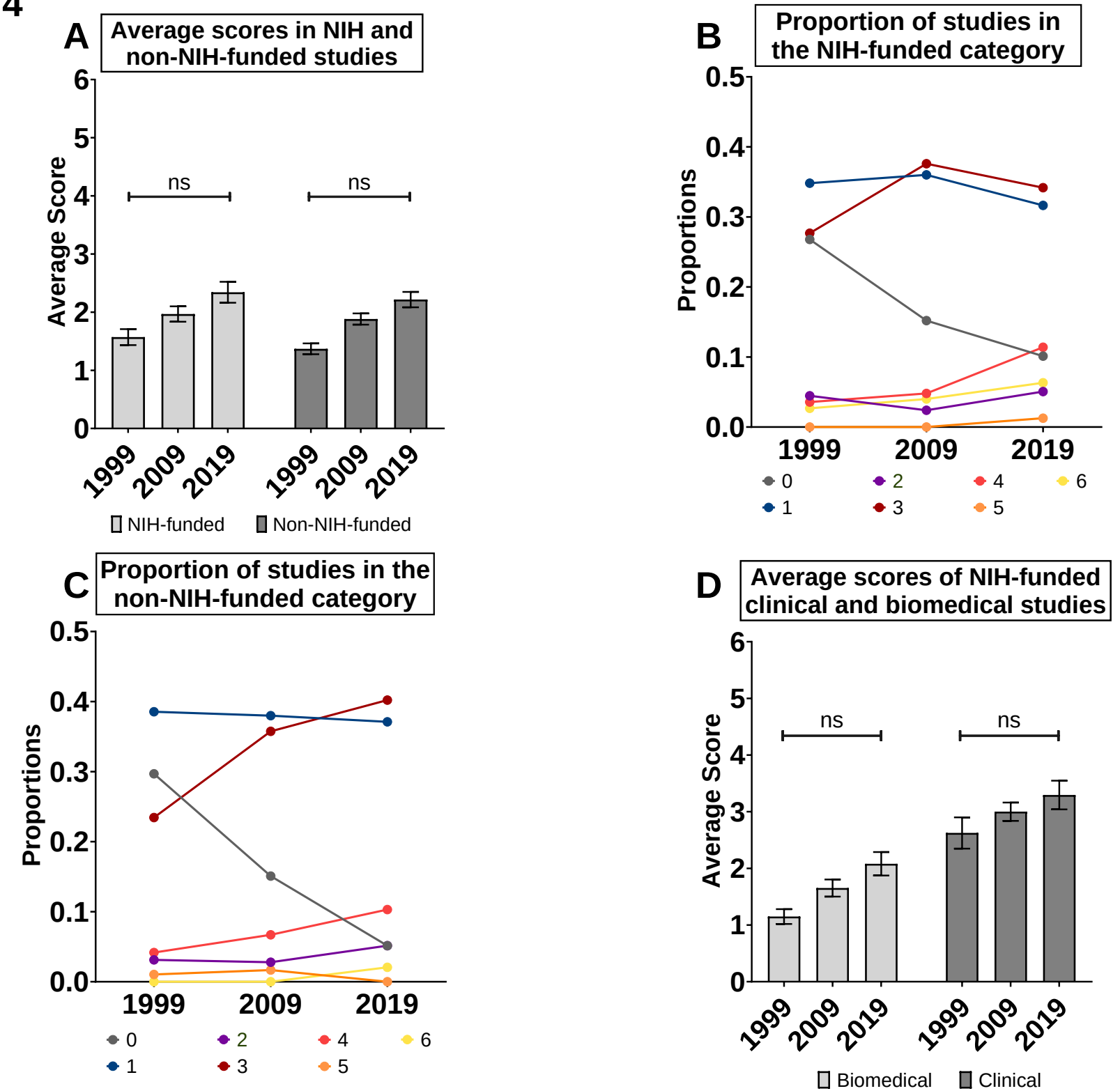
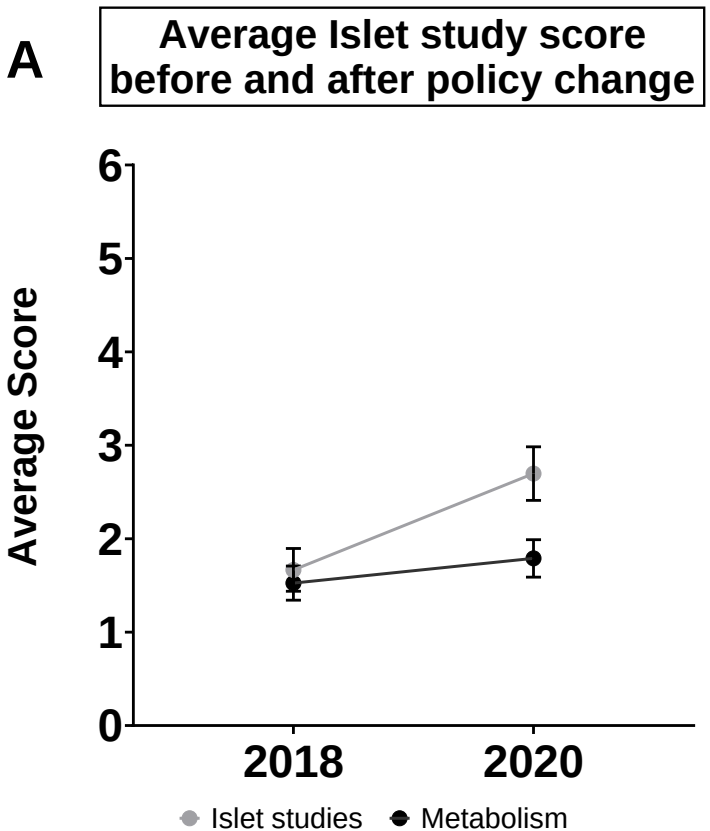
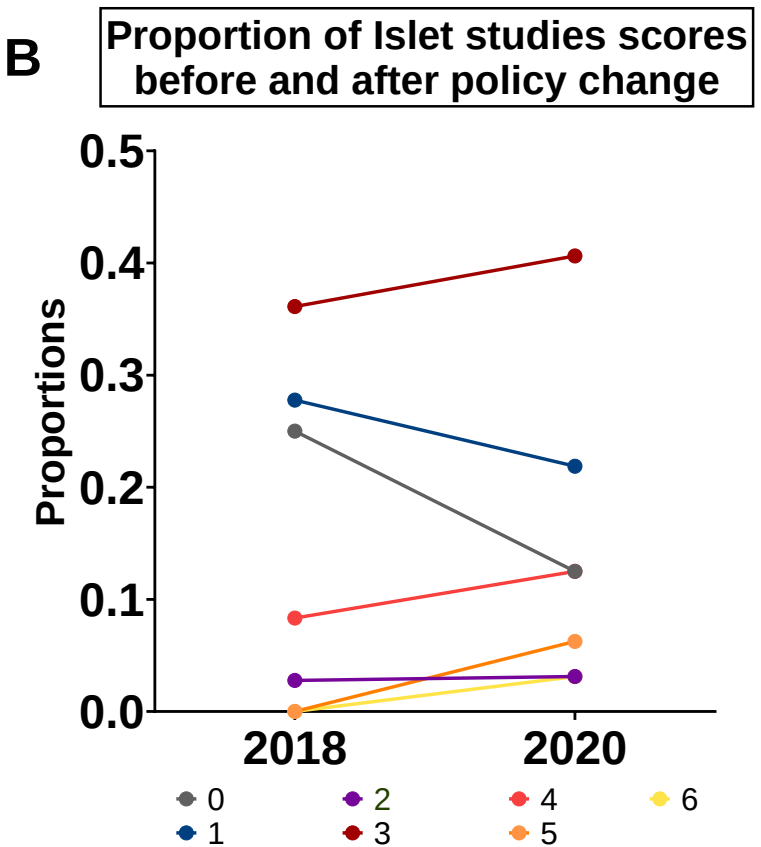

A
Average scores in NIH and non-NIH-funded studies
Average Score
ns
ns
1999
2009
2019
1999
2009
2019
NIH-funded
Non-NIH-funded
B
Proportion of studies in the NIH-funded category
Proportions
1999
2009
2019
0
1
2
3
4
5
6
C
Proportion of studies in the non-NIH-funded category
Proportions
1999
2009
2019
0
1
2
3
4
5
6
D
Average scores of NIH-funded clinical and biomedical studies
Average Score
ns
ns
1999
2009
2019
1999
2009
2019
Biomedical
Clinical

FIGURE 5


A
Average Islet study score before and after policy change
Average Score
6
5
4
3
2
1
0
2018
2020
Islet studies
Metabolism


B
Proportion of Islet studies scores before and after policy change
Proportions
0.5
0.4
0.3
0.2
0.1
0.0
2018
2020
0
1
2
3
4
5
6