Comment on ‘Exercise training decreases pancreatic fat content and improves beta cell function regardless of baseline glucose tolerance: a randomised controlled trial’. Reply to Amini P and Moharamzadeh S [letter]

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Abbreviations

DIA Diabetes status
PFC Pancreatic fat content

To the Editor: We thank Amini and Moharamzadeh for their comments and criticism [1] regarding our recently published article in *Diabetologia* [2]. In this article, we investigated the effects of exercise training on pancreatic fat content (PFC) and beta cell function in healthy men, and in men and women with prediabetes (impaired fasting glucose and/or impaired glucose tolerance) or type 2 diabetes [2].

To address the first comment by Amini and Moharamzadeh [1], when comparing the effects of exercise training in healthy individuals and those with prediabetes or type 2 diabetes, only men were included in our analysis because the healthy participants in our study were all men. Hence, we did not want to mix the effects of glucose intolerance (prediabetes or type 2 diabetes) with the effects of sex, as explained in our published article [2]. We admit that this is not optimal, but given the small number of participants that we had in each participant

group [2], we could not include sex as an additional factor in the analysis between healthy individuals and individuals with prediabetes/diabetes. Nonetheless, the results regarding the effects of exercise training on glycaemic control, pancreatic metabolism and beta cell function in both men and women with prediabetes and diabetes are presented in the electronic supplementary material (ESM) Tables 2–4 published with our article [2].

Regarding the second point by Amini and Moharamzadeh [1], it appears that there is a misunderstanding in the interpretation of the statistics of our study. Therefore, we would like to explain the analysis in more detail.

In their letter [1], Amini and Moharamzadeh outlined the model they believed was used in our study, which was previously described by Fitzmaurice and colleagues [3]. In this model, time is considered as a continuous numerical factor and the statistical inference is based on the regression slopes, describing linear change over the study period, as with normal linear regression. In our study, however, the timing for all repeated measures was common for all individuals (before and after the training intervention). Moreover, in our model, the mean response is estimated at each time point, stratified by group. Our model is indeed described in the book by Fitzmaurice et al, but in a different chapter to the one cited by Amini and Moharamzadeh [3]. In this chapter, with regards to the time effect, it is stated ‘hypothesis concerning the main effect of time translate into questions concerning whether the overall (i.e. averaged over groups) mean response has changed from baseline’ [3].

More precisely, in our study the analysis was performed with a hierarchical linear mixed model with one between-subject variable (diabetes status [DIA]) and one within-

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Table 1 Type 3 tests of fixed effects

Effect	Num DF	Den DF	<i>F</i> value	Pr > <i>F</i>
DIA	1	38.9	7.24	0.0104
Pre_post	1	29.9	4.86	0.0355
DIA*Pre_post	1	29.9	0.43	0.5183

p values for the main effects (DIA and pre_post) and their interaction (DIA*pre_post) are shown

Den DF, degrees of freedom of the denominator; Num DF, degrees of freedom of the numerator; Pr > *F*, two-tailed significance probability

subject variable (training effect, pre_post), and their interaction (DIA*pre_post). In this model, the interaction term is checked first. If it is significant, it means that the training effect differs between the groups of healthy men and men with prediabetes/diabetes. As the interaction term was not significant with regards to PFC in our analysis ($p = 0.5183$; see Table 1), it means that the training effect (if there was any) did not differ between the groups.

We then analysed the main effects (DIA and pre_post). For PFC, they were both significant (see Table 1). A statistically significant main effect for DIA means that PFC was different between the healthy men and men with prediabetes/diabetes, both pre and post intervention (because the interaction [DIA*pre_post] was not significant). A statistically significant main effect for pre_post means that PFC was different between pre and post exercise measurements, for both healthy men and men with prediabetes/diabetes (again, because the interaction was not significant). From the estimates in Table 2, we can see the direction of the change, with PFC being shown to decrease after training.

```
PROC MIXED data=dat2 plots=studentpanel;
WHERE sex=1;
CLASS id DIA pre_post;
MODEL sqrt_PFC = DIA pre_post DIA*pre_post /DDFM=KR;
REPEATED pre_post/SUBJECT=id TYPE=cs;
LSMEANS pre_post DIA*pre_post/DIFF CL;
RUN;
```

Fig. 1 SAS code used for the analysis of effects of exercise training on PFC and beta cell function in healthy men and men with prediabetes/diabetes in [1]

The SAS code that was used for analysing the training effect in our study [2] is presented in Fig. 1. ‘Sex = 1’ indicates that only men were included in the analysis, DIA indicates diabetic status (healthy group, DIA = 1; prediabetes/type 2 diabetes group, DIA = 2), and pre_post values indicate time of exercise intervention (before the intervention, pre_post = 1; after the intervention, pre_post = 2).

The square root-transformed PFC values are shown in Table 2. From these estimates we can see that before the exercise intervention the PFC value for DIA group 1 (healthy men) was 2.1000, whilst the pre-intervention PFC value for DIA group 2 (men with prediabetes/type 2 diabetes) was 2.9464. On average, the PFC value for both groups before the intervention was 2.5232.

In comparison, post intervention, the PFC value for DIA group 1 was 1.9089, whilst the post value for DIA group 2 was 2.5942, and the average for both groups post intervention was 2.2516 (Table 2).

The estimate of the difference between the two time points (pre_post) is shown in Table 3. This estimate is exactly the difference between 2.5232 and 2.2516 ($2.5232 - 2.2516 = 0.2716$), resulting in the same *p* value ($p = 0.0355$) as in the table for type 3 tests of fixed effects (Table 1).

Table 2 Least squares mean

Effect	DIA ^a	Pre_post ^b	Estimate	Standard error	DF	<i>t</i> value	Pr > <i>t</i>	Alpha	Lower	Upper
DIA	1		2.0044	0.1686	39.1	11.89	<.0001	0.05	1.6635	2.3454
DIA	2		2.7703	0.2293	38.8	12.08	<.0001	0.05	2.3064	3.2342
Pre_post		1	2.5232	0.1545	50.3	16.34	<.0001	0.05	2.2130	2.8333
Pre_post		2	2.2516	0.1557	51.2	14.46	<.0001	0.05	1.9390	2.5641
DIA*Pre_post	1	1	2.1000	0.1818	49.8	11.55	<.0001	0.05	1.7347	2.4652
DIA*Pre_post	1	2	1.9089	0.1860	52.3	10.26	<.0001	0.05	1.5357	2.2822
DIA*Pre_post	2	1	2.9464	0.2497	50.6	11.80	<.0001	0.05	2.4449	3.4478
DIA*Pre_post	2	2	2.5942	0.2497	50.6	10.39	<.0001	0.05	2.0928	3.0956

Square root-transformed PFC values are shown. Data are presented as model-based least square means

^a Healthy group, DIA = 1; prediabetes/type 2 diabetes group, DIA = 2

^b Before the intervention, pre_post = 1; after the intervention, pre_post = 2

DF, degrees of freedom; Pr > |*t*|, *p* value for the two-tailed probability computed using the *t* distribution

Table 3 Differences of least squares means

Effect	DIA ^a	Pre_post ^b	DIA ^a	Pre_post ^b	Estimate	Standard error	DF	<i>t</i> value	Pr > <i>t</i>	Alpha	Lower	Upper
Pre_post		1		2	0.2716	0.1232	29.9	2.20	0.0355	0.05	0.01986	0.5233
DIA	1		2		−0.7658	0.2846	38.9	−2.69	0.0104	0.05	−1.3416	−0.1901

^a Healthy group, DIA = 1; prediabetes/type 2 diabetes group, DIA = 2

^b Before the intervention, pre_post = 1; after the intervention, pre_post = 2

DF, degrees of freedom; Pr > |*t*|, *p* value for the two-tailed probability computed using the *t* distribution

Therefore, the *p* value of 0.0355 for the main effect pre_post represents the mean change in PFC after training for both the healthy and prediabetes/diabetes group. The result is also presented graphically in Fig. 2b of the original publication, which indicates that PFC is decreased in both participant groups, with no difference between the groups.

Finally, we acknowledge that HbA_{1c} analysis reflects mean blood glucose over the preceding 2–3 months. The finding that exercise training decreases HbA_{1c} has been discussed in detail in our previous publication [4], in which we provide possible explanations for the observed exercise-induced decrease in HbA_{1c} in the participants with prediabetes or diabetes in our recent study in *Diabetologia* [2]. As discussed previously, the most probable explanation for decreased HbA_{1c} after such a short intervention is haemodilution, most likely due to increased plasma volume, which is typically observed after exercise [4]. HbA_{1c} values were included in our publication in *Diabetologia* [2] to provide background information for the participants and it was not discussed further to avoid dual publication of results. It is true that there are better biomarkers than HbA_{1c} to monitor blood glucose change in such a short training intervention study.

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