



UNIVERSITA' DEGLI STUDI DI VERONA

SCUOLA DI MEDICINA E CHIRURGIA
Dipartimento di Medicina

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Technical report describing the effect on blood glucose levels observed following ingestion of innovative bakery products made by Andrea Menegale as compared with traditional bakery products

1. Background

Carbohydrates are an indispensable part of the human diet because glucose is the main source of energy for the body and virtually the only substrate utilized by the brain (1). In order to provide the allowed amount of energy, any reduction in dietary carbohydrates is necessarily compensated by an increase in the proportion of lipids and/or proteins. This implies unfavorable effects in terms of risk of mortality as well as risk of cardiovascular and renal diseases (2, 3). For such reason, the World Health Organization recommends that around 50 per cent of the daily calorie intake should come from carbohydrates. However, the latter should include minimal amounts of simple sugars, especially sucrose, with complex sugars being used instead. The recommendation also suggests a higher intake of dietary fibers from sources like whole-grain cereals, legumes, vegetables and also, to a certain extent, nuts and fresh fruit (4).

Since a long time experts have been suggesting that foods with a lower glycemic index should be preferred in the composition of a diet, especially for people with diabetes. Such foods have in general a similar carbohydrate content but generate a smaller rise in blood glucose levels following their ingestion. Examples of these foods are basmati rice, rather than white rice, wholemeal bread, rather than white bread, and peaches, rather than watermelons (5). Diets with a lower glycemic load facilitate metabolic control and should be more widely consumed (6).

Bread and pasta are cornerstones of the Mediterranean diet and are also included, albeit in controlled quantities, in the nutritional plan of those suffering from diabetes (7). However, the carbohydrate and fiber content of bread and pasta, and of other bakery products such as breadsticks and biscuits, can vary considerably and therefore can have a substantially different impact on glycemic profiles of persons with diabetes. Yet, repeated exposure to an excessive increase in blood glucose after meal is known to yield detrimental effects on the function of insulin-producing cells and to convey an increased risk for the future development of diabetes in people without the disease. This knowledge prompted to the development of special foods labeled 'for diabetics' which generally have a higher fiber content and in which sucrose is often replaced by natural or artificial sweeteners. However, the presumed absence of detrimental effects by artificial sweeteners has recently been challenged by studies in which a sort of "diabetogenic" change in the intestinal microbiome was observed in animals and humans consuming these sweeteners (8).



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I have recently received a formal request, in the context of a specific agreement with my University Department, to express a technical opinion on the glycemc profiles observed in people with and without diabetes following the intake of traditional and alternative bakery products. The latter were produced using flour mixtures generally available on the market and without the addition of probiotics and were created with the purpose of slowing the digestion process and the subsequent absorption of carbohydrates. The technical opinion presented below comprises an evaluation of the methods employed and an interpretation of the results observed.

2. Products used for comparative testing

Four types of bakery products underwent a formal and standardized comparison: bread, pasta, breadsticks and biscuits. Comparison were conducted by testing traditional products (TPs) available on the market or innovative products (IPs) created in a private bakery. It should be noted that the TPs were of the wholemeal variety, i.e. not based on '00' or '0' type flour and therefore consistent with those mostly recommended by current guidelines which discourage the consumption of refined products. For creating IPs both soft wheat flour and durum wheat flour were used. There were, however, substantial differences in the degree of flour refining. Noteworthy, the grinding process involved crushing rather than pulverization in order to reduce the level of refining and hence slowing down the action of amylase on starch. There were also significant differences in the flour mixtures used to produce the IPs compared to the TPs.

Tables 1 to 4 summarize the characteristics of the TPs and the corresponding IPs. The differences in terms of energy (kcal) were minimal, if any. It is important to emphasize that in all the IPs the quantity of simple carbohydrates, or sugars, was limited (1 to 3 g) and quite similar to the TPs, whereas the total quantity of carbohydrates sometimes moderately differed: slightly more in the innovative pasta (+10%) and slightly less in the innovative breadsticks (-11%) and the innovative biscuits (-10%). The latter contained more proteins (+75%) while in the case of the other products there were no differences or only minimal differences in protein content between TPs and IPs (+10% in traditional pasta and +6% in innovative breadsticks). The total lipid content did not differ in the two types of bread and biscuits, while it was higher in traditional vs. innovative pasta (+39%) and in innovative vs. traditional breadsticks (+48%). The quantity of fiber in the IPs was not greater, but in some cases actually smaller (-31% in pasta, -11% in breadsticks and -7% in biscuits).



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3. Study population

The study was performed on a sample of around 150 people, all of whom volunteered. Each comparison included all subjects or separately subjects with or without type 2 diabetes.

Tables 5 to 8 summarize the characteristics of subjects examined in each comparison. Each subject underwent two tests, one with the TP and one with the IP. The tests were carried out on different days and always in the late afternoon (around at 6 pm). They took place in random order after at least four hours of fasting. Subjects with diabetes were asked not to change their habitual therapy in the day of testing. None of them were being treated with insulin. Diabetic persons on anti-hyperglycemic drugs were requested to refrain from taking them before the test as the food ingested did not constitute a complete meal.

Glycemic test

The glucose in the capillary blood of the subjects was measured by means of a glucose meter before and then 30, 60 and 90 minutes after ingestion of the food. The food was consumed on its own without any accompaniment over a period of five to ten minutes. In all tests, the TP and the IP were ingested in the same quantity (weighed using a digital scale): 50 g for bread, 60 g for pasta, 50 g for breadsticks and 50 g for biscuits. The pasta was cooked in both cases for about ten minutes and consumed after being seasoned with extra virgin olive oil (one tablespoon).

All blood glucose measurements were performed using the same type of glucose meter (Verio Flex, Lifescan). Blood samples were taken by health professionals observing all the standard precautions: washing the hand of the subject to be examined and avoiding the use of disinfectant so as to exclude any possibility of contamination or dilution of the capillary blood.

5. Statistics

Comparison of glucose levels or glycemic parameters after meals based on TPs or IPs was analyzed by Student's *t* test for paired data. Also two-way analysis of variance for repeated measurements (ANOVA) were used. The data are presented in figures as mean±standard deviation.



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6. Results

Bread

As documented by the ANOVA, the glycemc curve during bread-based meal was significantly different in all subjects and in those with diabetes. The values were lower with the IP compared to the TP. Significant differences in the glycemc levels were observed at 60 and 90 minutes, such values being lower with the IP compared to the TP in all subjects and, to a greater extent, in those with diabetes (Figures 1 to 3).

The glycemc peak (the maximum value reached during the test) was lower with the IP in all subjects and in those with diabetes (Figure 4).

The absolute blood glucose increase vs. baseline was smaller with the IP compared to the TP in all subjects and, with borderline statistical significance, in those with diabetes (Figure 5).

Despite a clear trend toward lower values with the IP, the absolute and the incremental areas under the glycemc curves were not significantly different in all subjects or in subgroups with or without diabetes (Figures 6 and 7).

Pasta

The glycemc curve during pasta-based meal was not significantly different with the IP compared to the TP (ANOVA not significant). However, significantly lower glycemc levels were observed at 60 and 90 minutes with the IP in subjects with diabetes (Figures 8 to 10).

The glycemc peak was significantly lower after the IP compared to the TP in subjects with diabetes (Figure 11).

The absolute blood glucose increase vs. baseline was substantially smaller with the IP in subjects with diabetes but the difference did not reach statistical significance (Figure 12).

The absolute and the incremental areas under the glycemc curve were significantly lower in subjects with diabetes with the IP vs. the TP (Figures 13 and 14).



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Breadsticks

The glycemc curve during meal based on breadsticks was significantly lower after the IP vs. the TP both in subjects with and without diabetes but not in the whole sample. Lower glycemc levels were observed with the IP vs. the TP. This was particularly true at 30 and 60 minutes in all subjects and in those without diabetes. Even in subjects with diabetes, blood glucose levels were lower 60 and 90 minutes after the IP but the differences did not reach statistical significance because the number of subjects was small (Figures 15 to 17)

The glycemc peak was significantly lower with the IP in all subjects and in those without diabetes (Figure 18).

The absolute blood glucose increase vs. baseline was statistically lower with the IP in all subjects and in those without and with diabetes. In the latter group, the peak was almost halved with the IP vs. the TP (Figure 19).

The absolute area under the glycemc curve was significantly lower with the IP vs. the TP in all subjects and in those without diabetes (Figure 20). The incremental area was significantly lower also in subjects with diabetes in whom it was approximately halved with the IP vs. the TP (Figure 21).

Biscuits

The glycemc curve during meal based on biscuits was significantly lower after the IP than after the TP both in all subjects and in those without diabetes. In all subjects and in those without diabetes lower glycemc values were observed with the IP vs. the TP after 60 and 90 minutes, whereas in the subjects with diabetes the difference was observed at 90 minutes (Figures 22 to 24).

The glycemc peak was significantly lower with the IP vs. the TP in all subjects and in those with and without diabetes (Figure 25).

The absolute blood glucose increase vs. baseline was lower with the IP vs. TP in all subjects and in those without diabetes, whereas in those with diabetes the difference, although substantial, was not statistically significant (Figure 26).

The areas under the glycemc curves (both absolute and incremental) were significantly lower with the IP vs. the TP only in subjects without diabetes (Figures 27 and 28).



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7. Discussion

One of the aims in the management of type 2 diabetes is to control the increase in blood glucose levels after meals. In fact, a bulk of scientific evidence supports the concept that postprandial glucose peaks have harmful effects on the arterial wall and are associated with the development of cardiovascular diseases (9). Furthermore, excessive increases in blood glucose levels, which are frequent in type 2 diabetes (10), lead to an increase in HbA1c and hence in the risk of chronic complications in the diabetes (11).

The nutritional plan of persons suffering from diabetes must inevitably include around 50% of carbohydrates, with current guidelines recommending a drastic limitation in simple sugars, a marked increase in consumption of fibers and a preference for foods with a lower glycemic index (6). The latter mainly include vegetables, legumes, nuts, fresh fruit and whole-grain cereals, in other words the basic components of the Mediterranean diet which indeed has also showed a benefit in primary cardiovascular prevention (12).

The meals of a person with diabetes cannot exclude a certain amount of bread and pasta or other bakery products such as breadsticks and biscuits. Bread, pasta, breadsticks and biscuits can be produced using flour with varying degrees of refining. The level of refining of flour influences its physical-chemical composition which in turn can make the food more or less accessible to enzymes promoting digestion and subsequent absorption. If complex carbohydrates (e.g. starch) are less accessible to the enzymes (mainly amylase), there is a reduction in the quantity of disaccharides available at the level of the intestinal brush and consequently in the quantity of monosaccharide absorbed, including glucose. The final result is a smaller increase in blood glucose levels after the ingestion of food in which the starch is less accessible.

The aim of making the starch less accessible to enzymes was fundamental to the creation of the innovative bakery products here used for comparisons with traditional products.

All the four innovative products evaluated in this study have been shown to yield smaller increases in blood glucose levels when compared with traditional products. This phenomenon was more evident in the case of breadsticks and biscuits since in these foods only innovative flour was used, while in the production of bread and pasta a mixture of innovative and traditional flour was created. It should also be noted that in the case of bread, pasta and biscuits the favorable effect on blood glucose was observed during the latter phase of the test (namely after 60 and 90 minutes), whereas for breadsticks it was observed in subjects without diabetes at an earlier stage (i.e. after 30 and 60 minutes).

Overall, these results suggest that the production of foods with a lower glycemic index can be based on different milling processes and on the use of flour mixtures different from those traditionally employed without any loss of the organoleptic properties of the food product.



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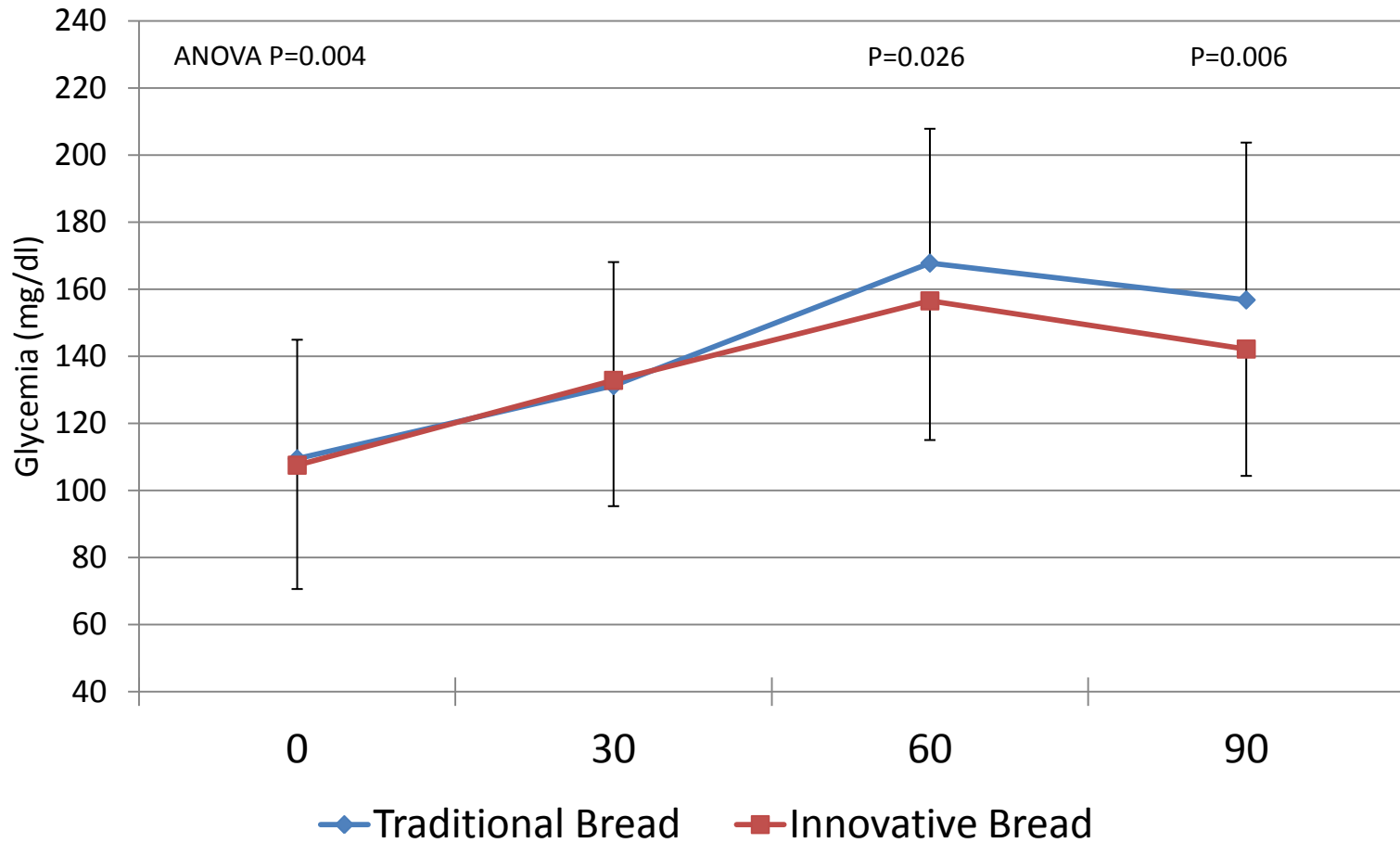
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Faithfully.

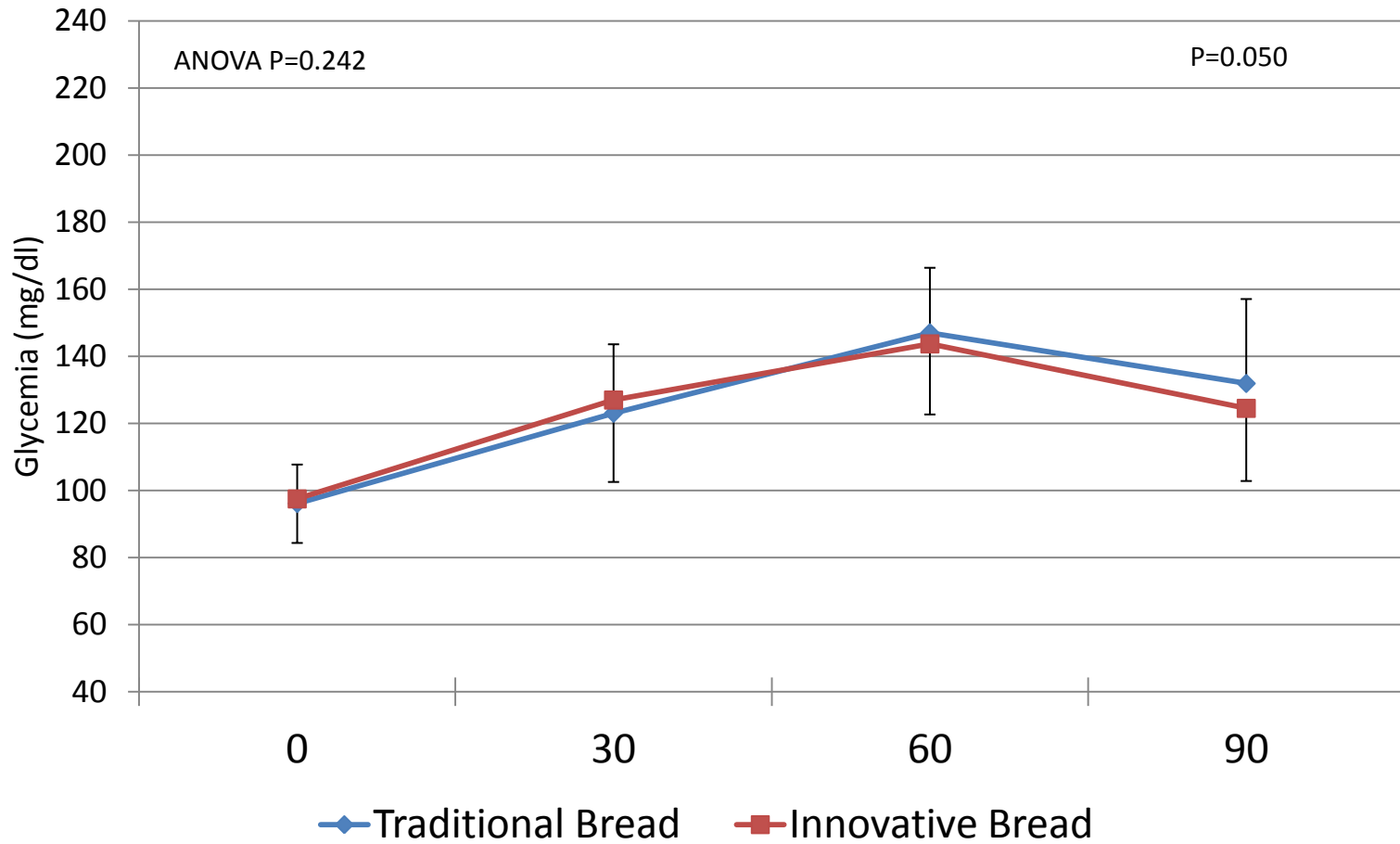
Enzo Bonora

A handwritten signature in black ink, appearing to read 'Enzo Bonora'.

Fig. 1 - Glycemic curve after a bread-based meal
All subjects (n = 35)



**Fig. 2 - Glycemic curve after a bread-based meal
Subjects without DM (n = 19)**



**Fig. 3 - Glycemic curve after a bread-based meal
Subjects with DM (n = 16)**

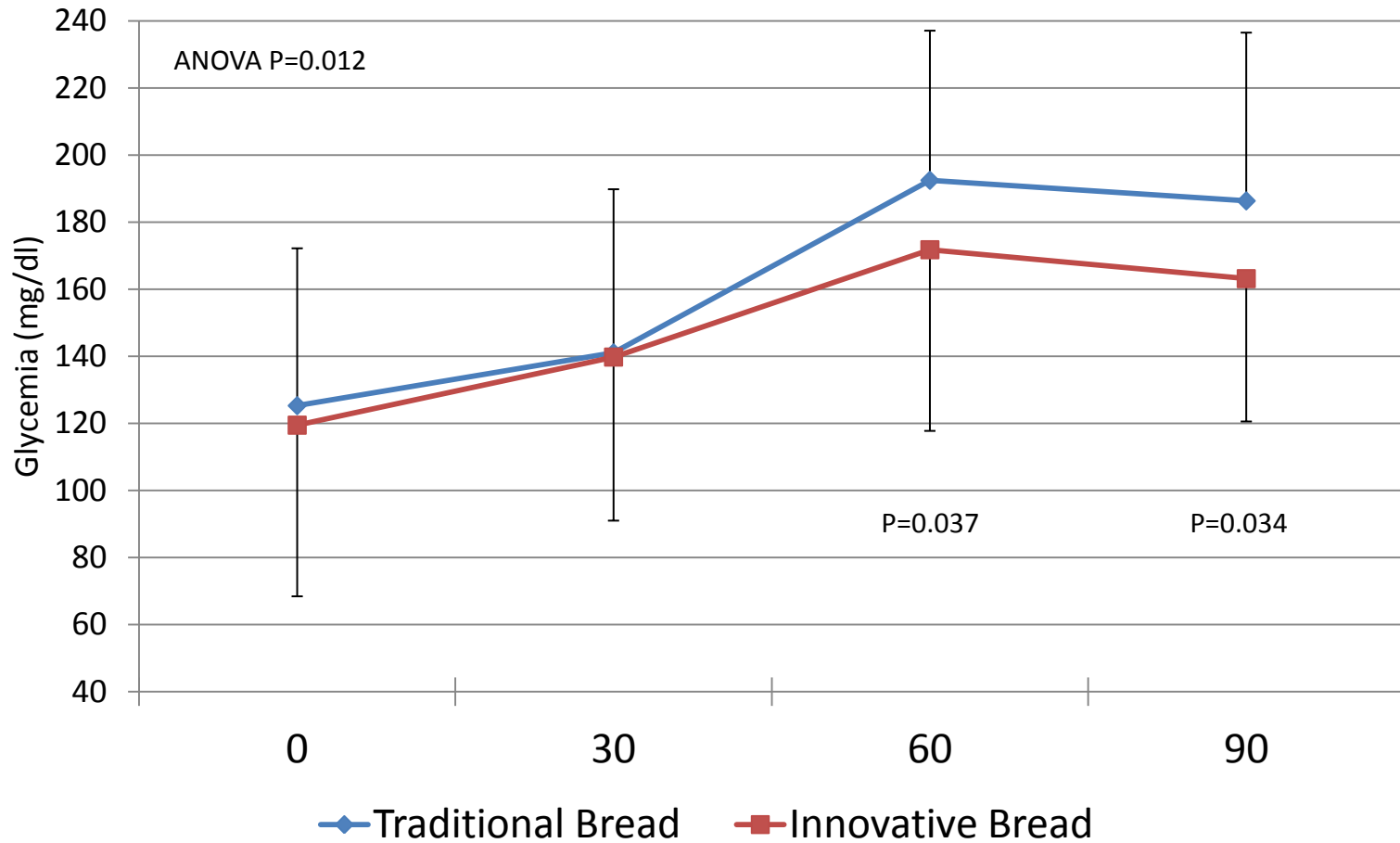


Fig. 4 - Glycemic peak after a bread-based meal

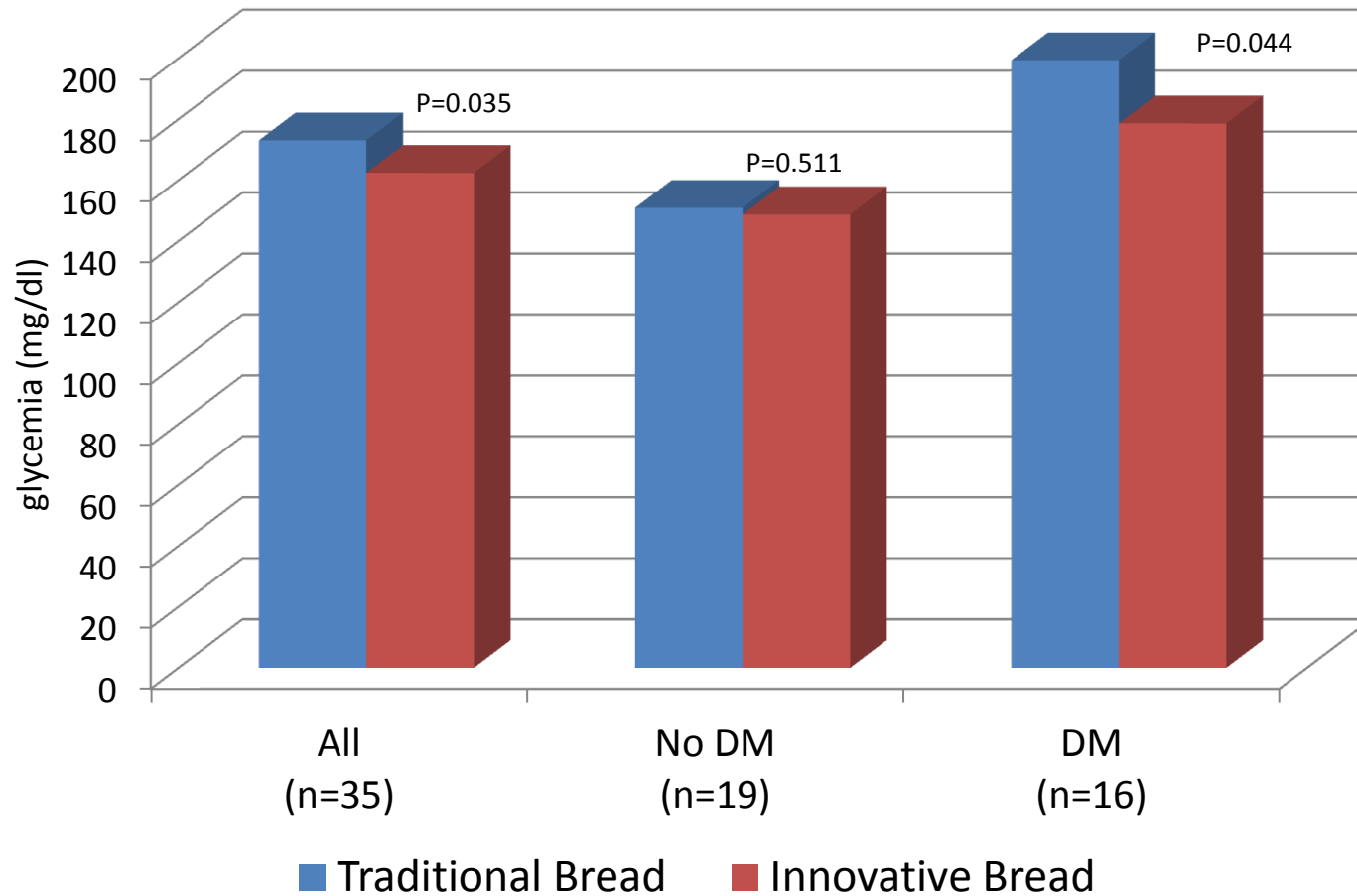


Fig. 5 - Blood glucose increase vs. baseline after bread-based meal

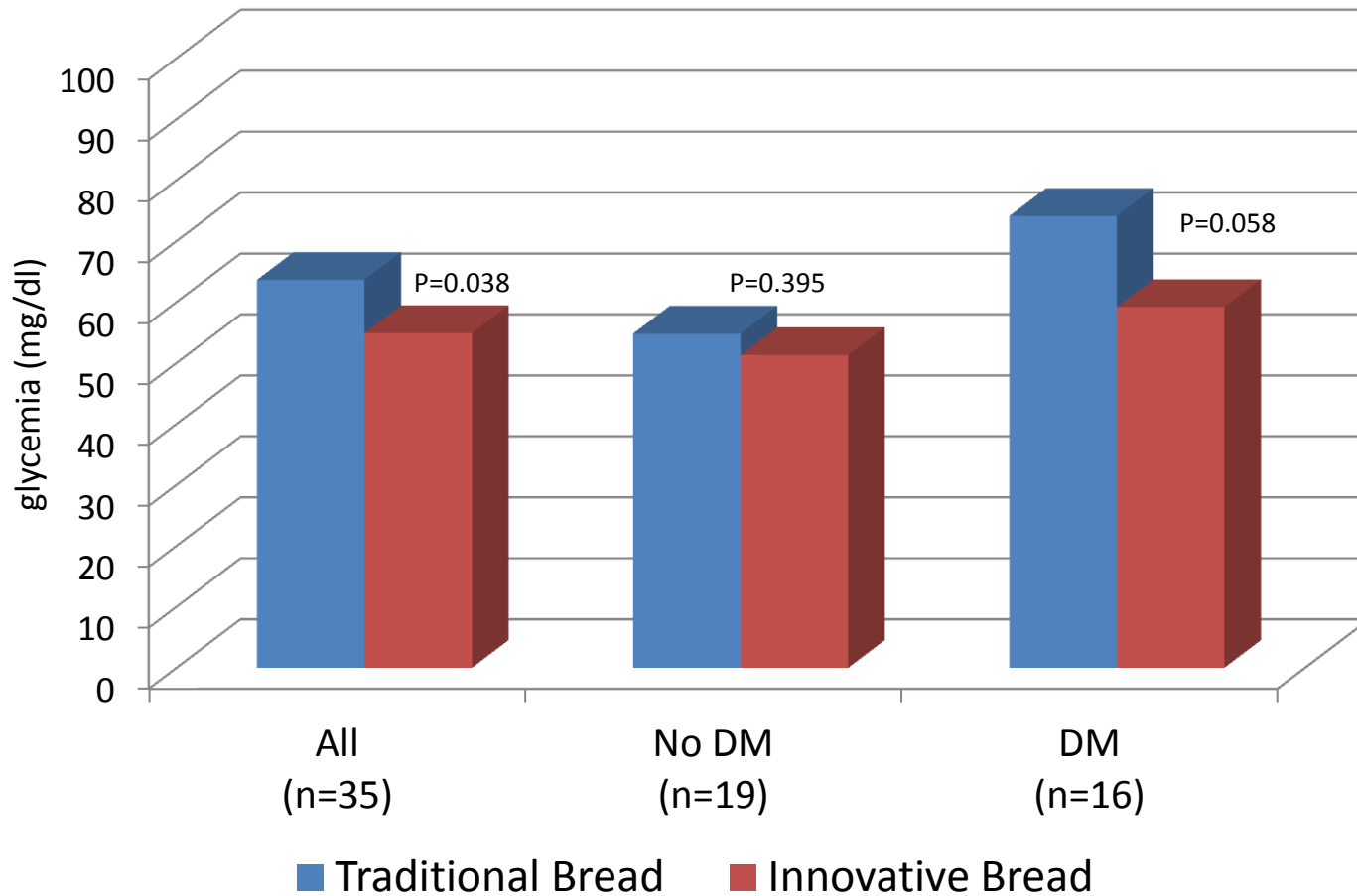


Fig. 6 - Area under the glycemic curve after a bread-based meal

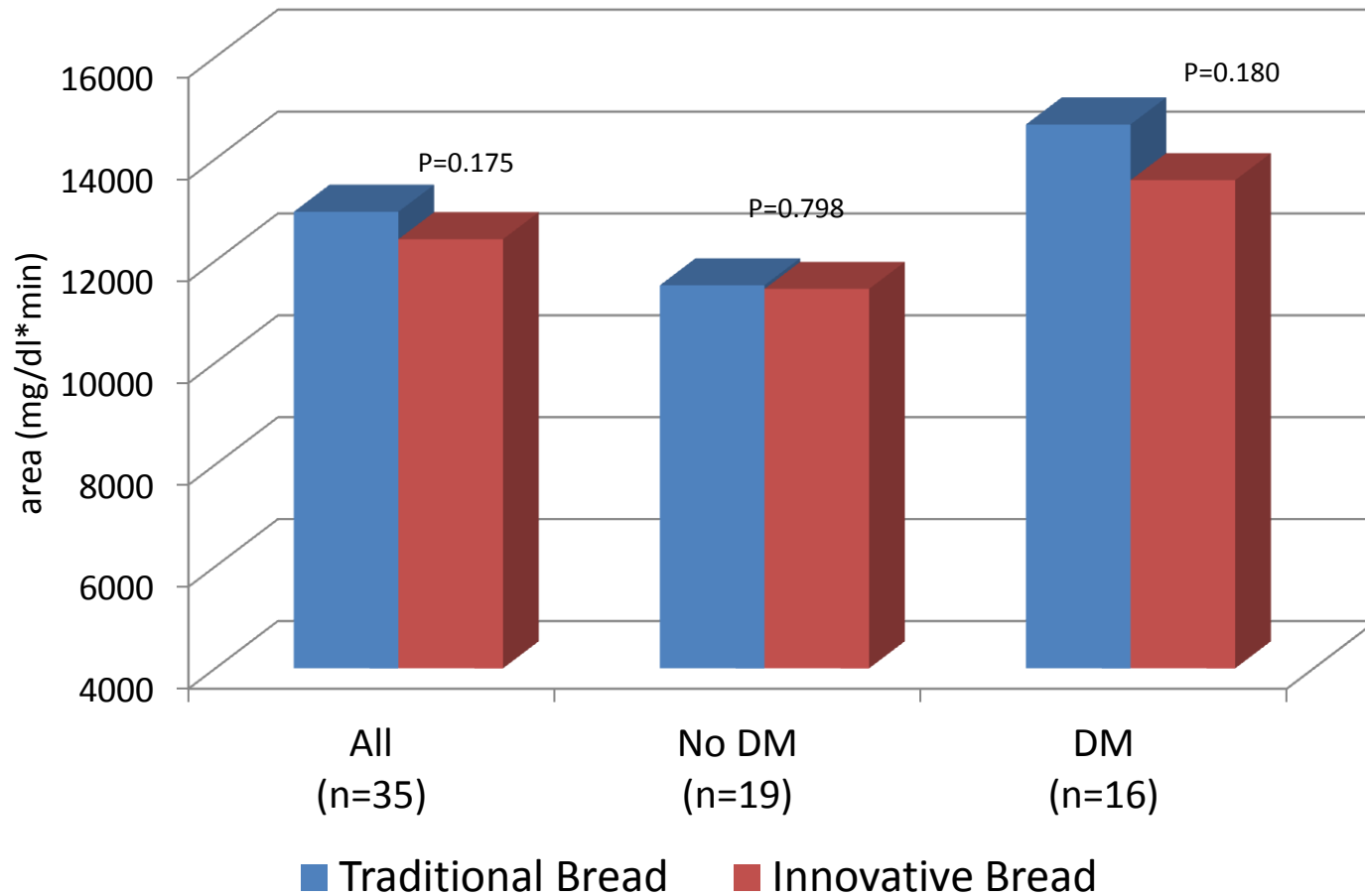


Fig. 7 - Incremental glyceimic area after a bread-based meal

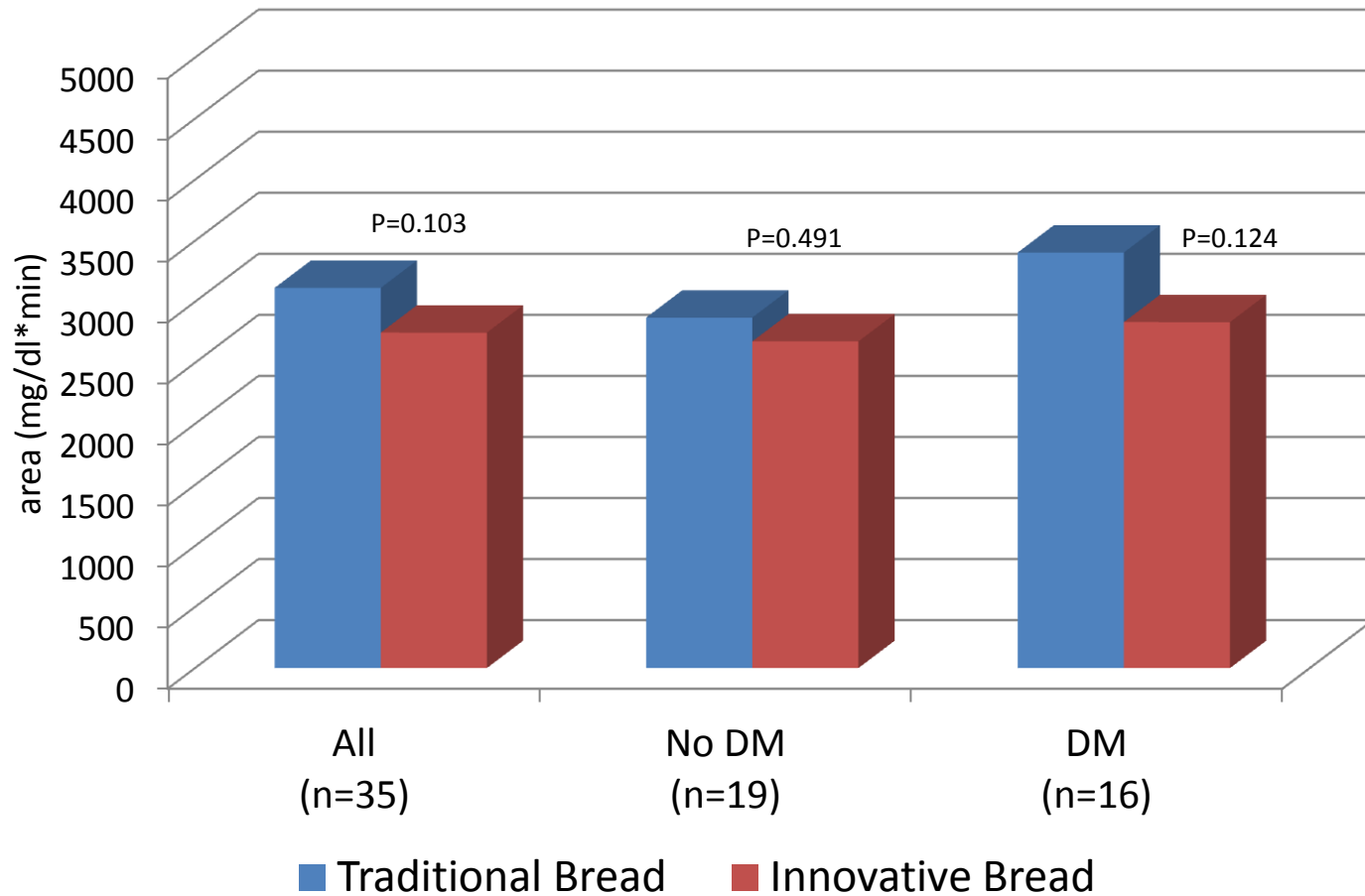


Fig. 8 - Glycemic curve after a pasta-based meal
All subjects (n = 48)

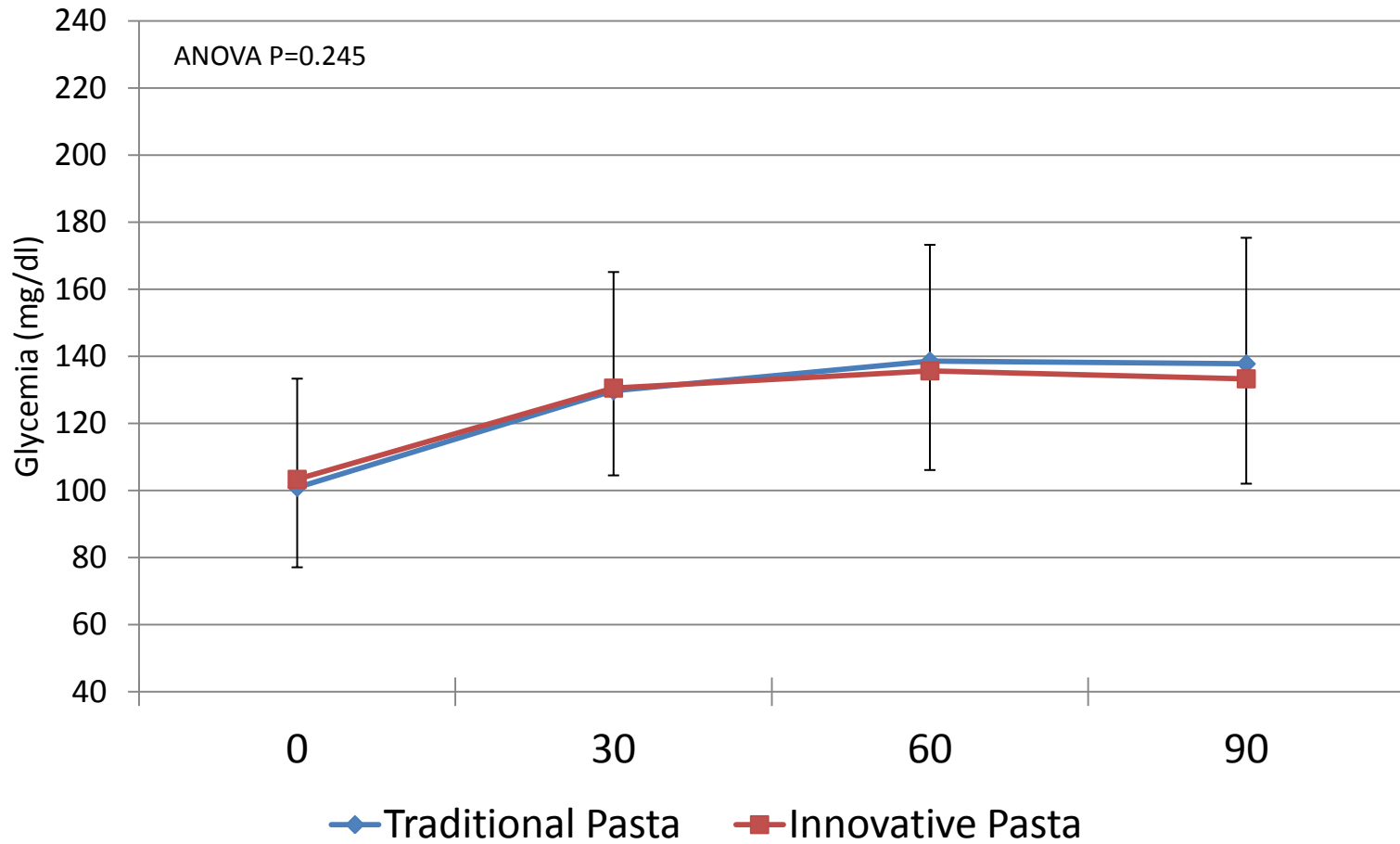
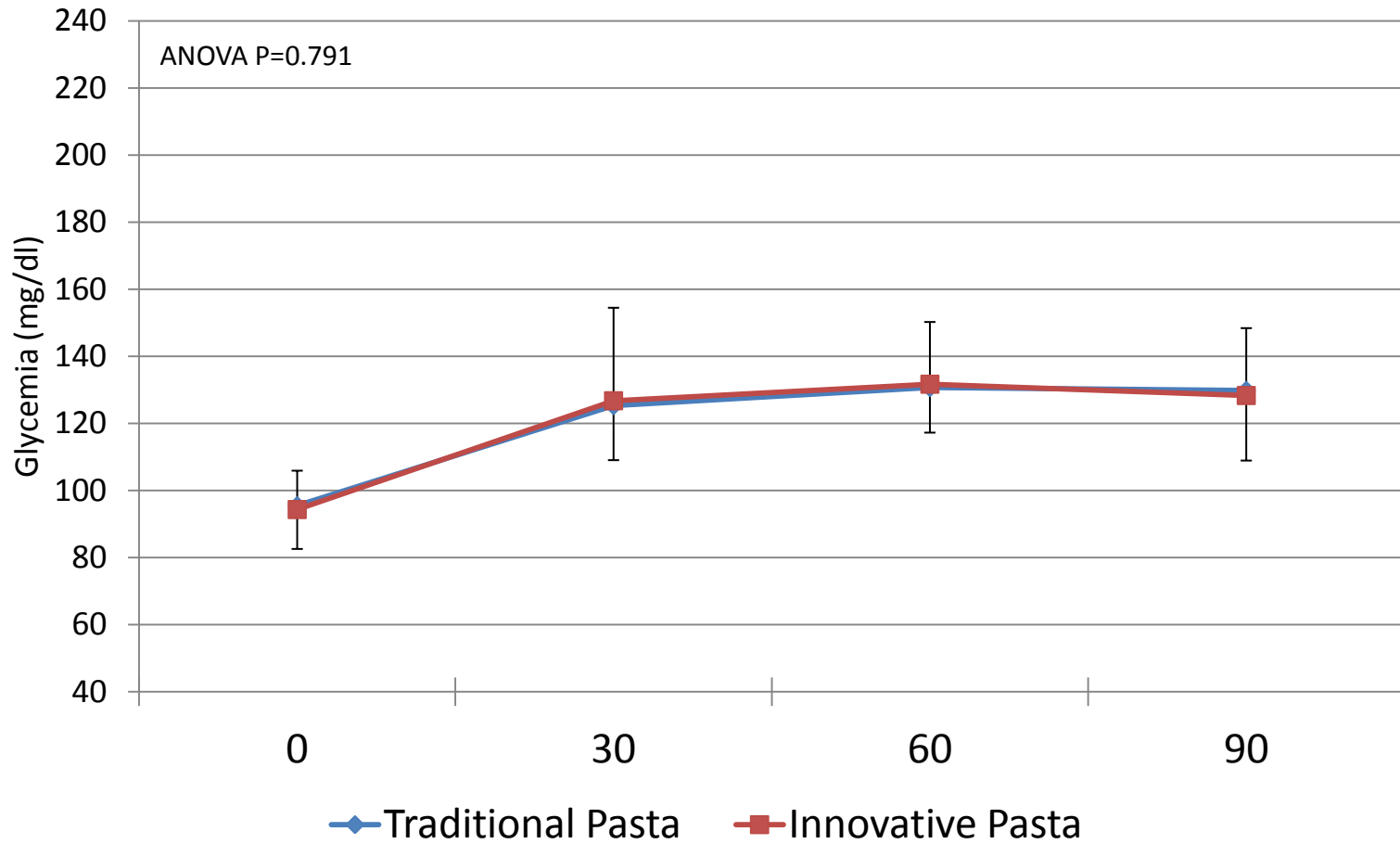


Fig. 9 - Glycemic curve after a pasta-based meal
Subjects without DM (n = 30)



**Fig. 10 - Glycemic curve after a pasta-based meal
Subjects with DM (n = 18)**

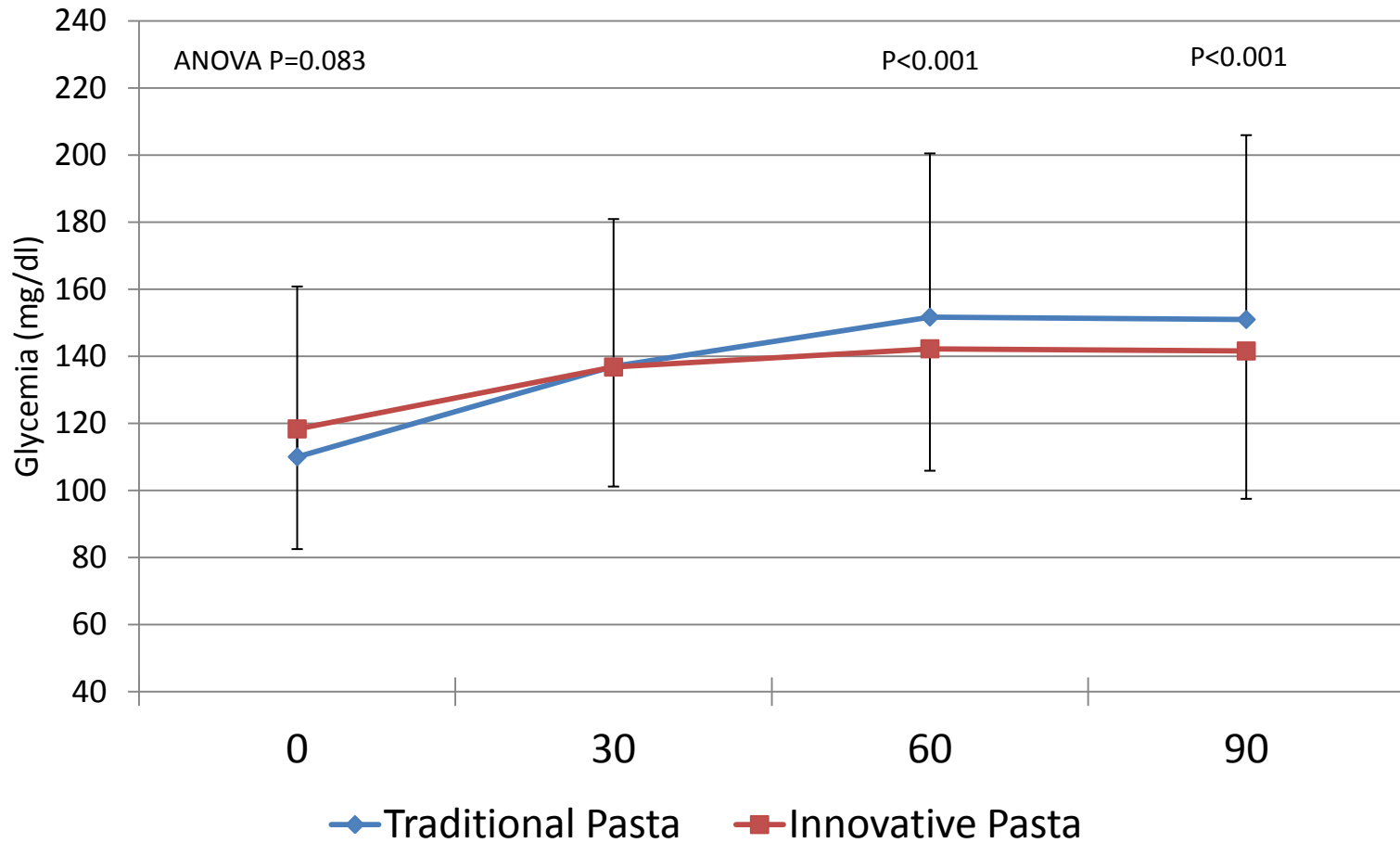


Fig. 11 - Glycemic peak after a pasta-based meal

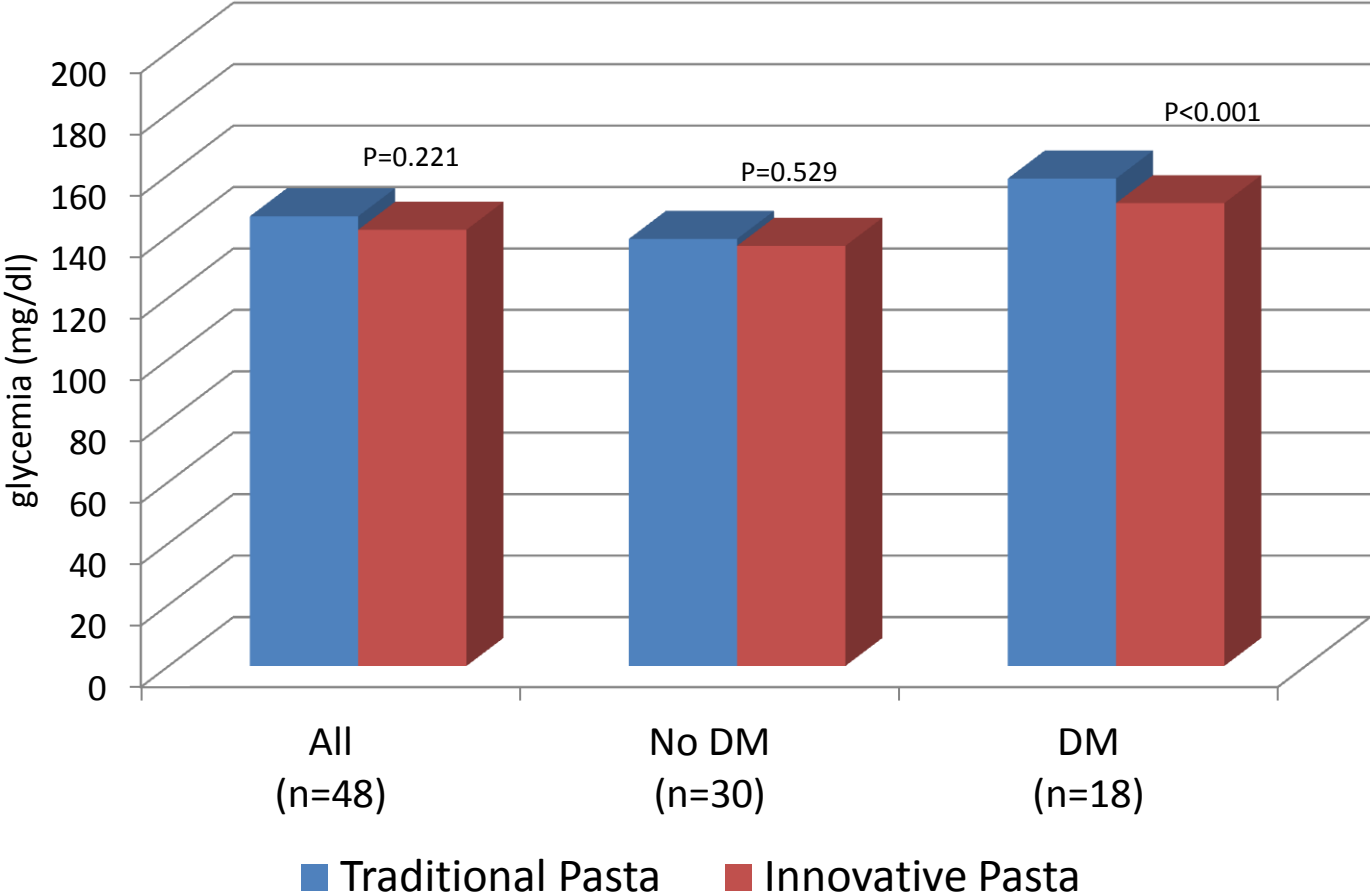


Fig. 12 - Blood glucose increase vs. baseline after a pasta-based meal

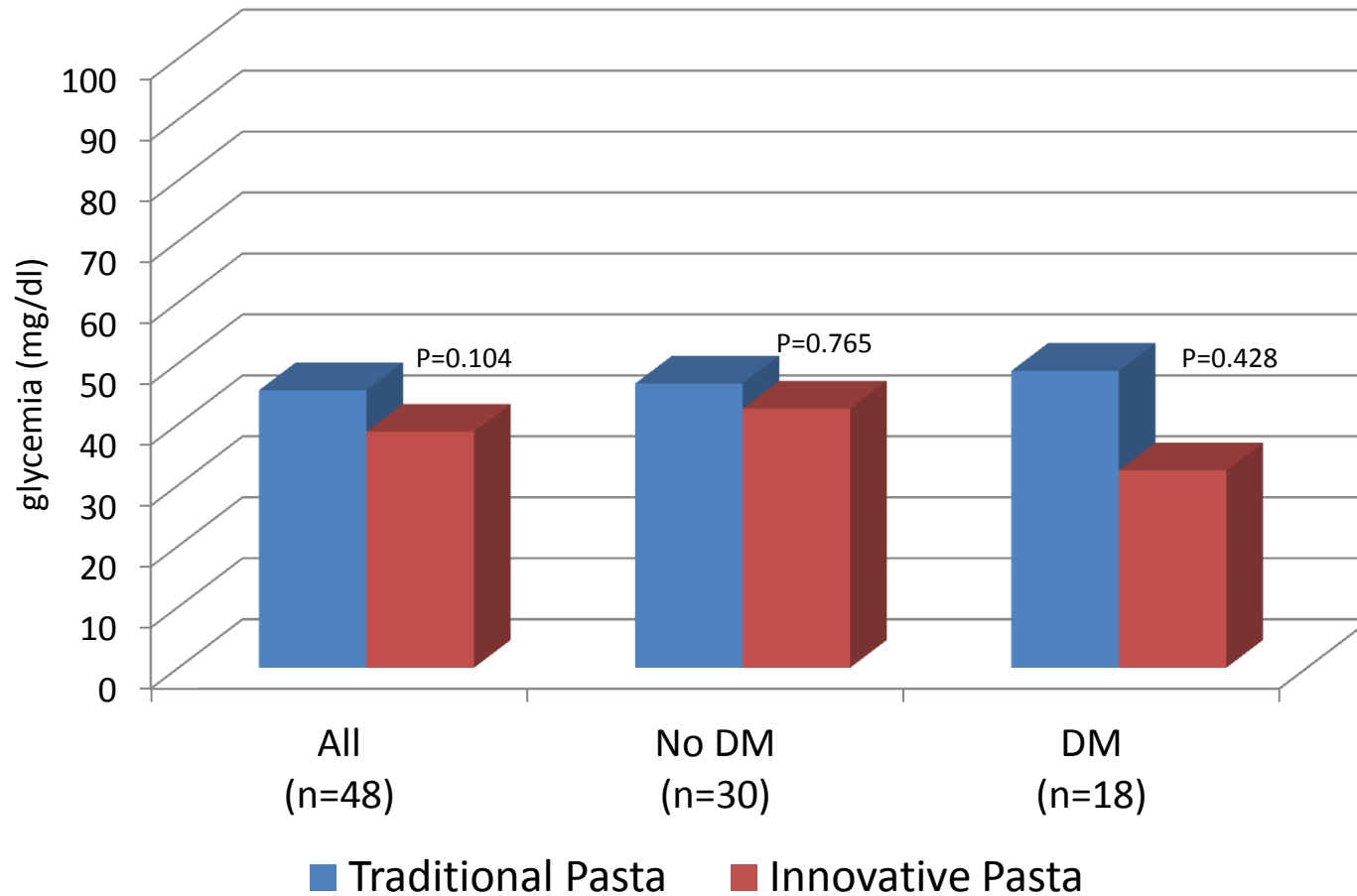


Fig. 13 - Area under the glycemic curve after a pasta-based meal

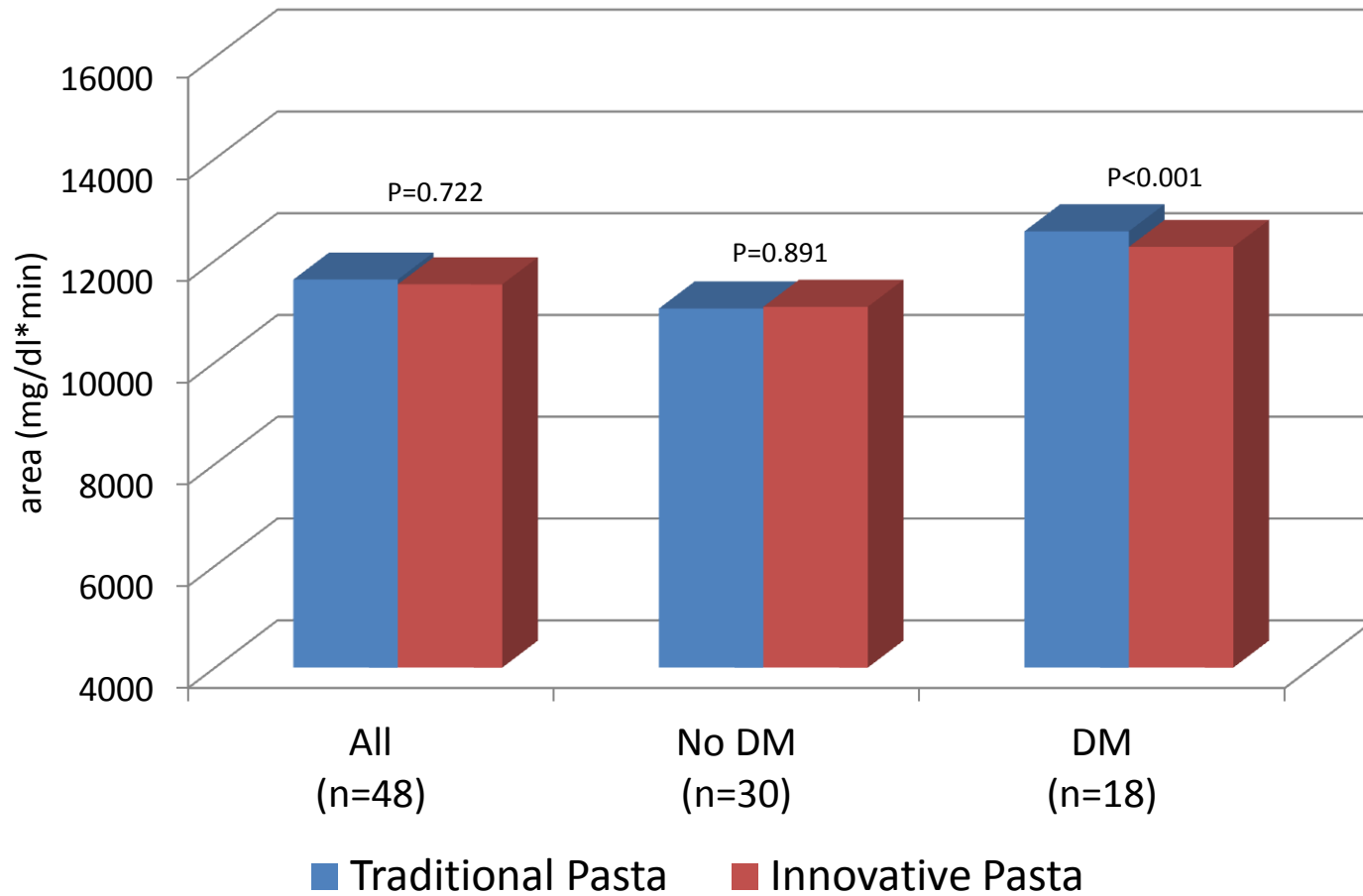


Fig. 14 - Incremental glyceimic area after a pasta-based meal

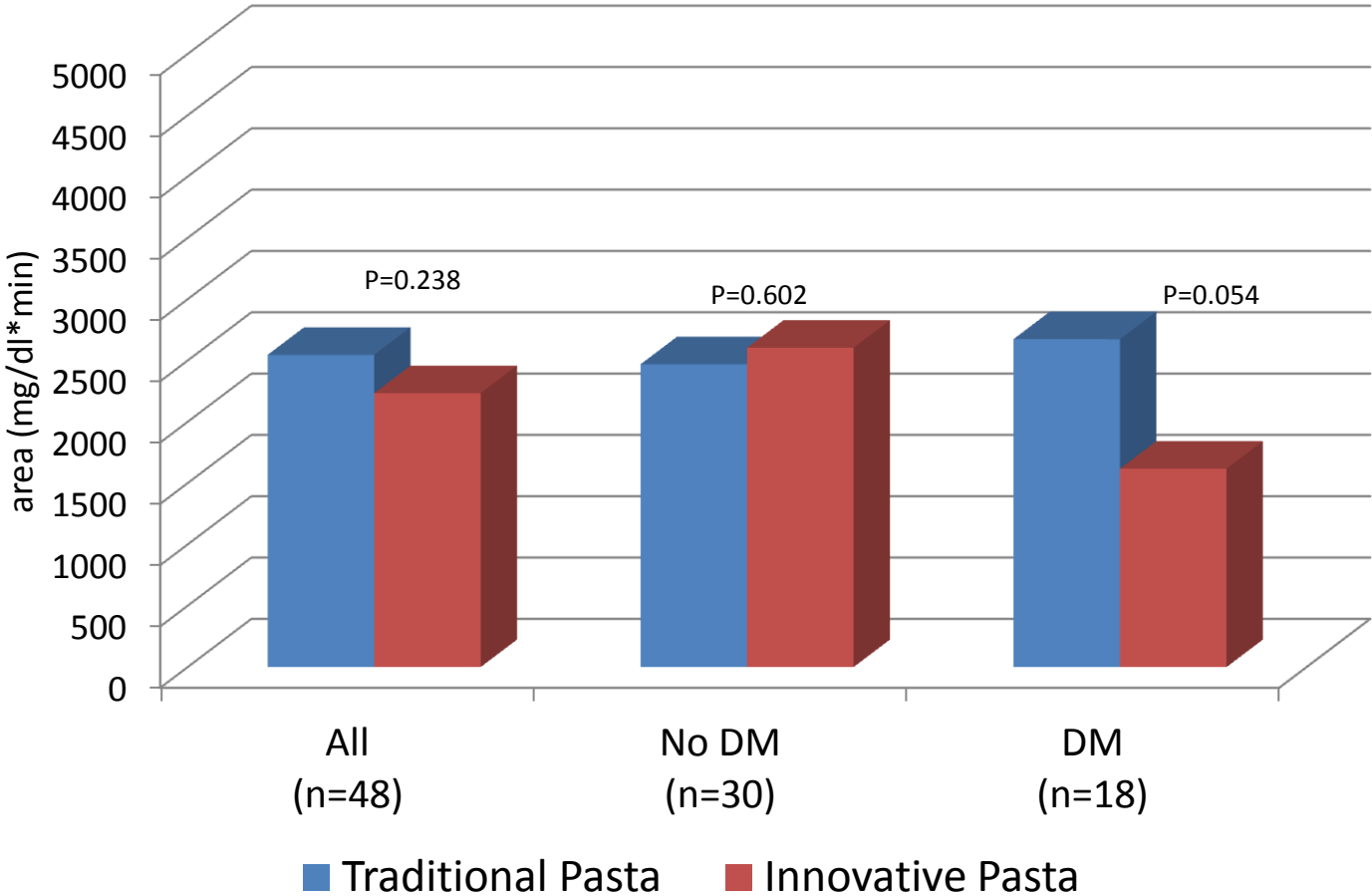
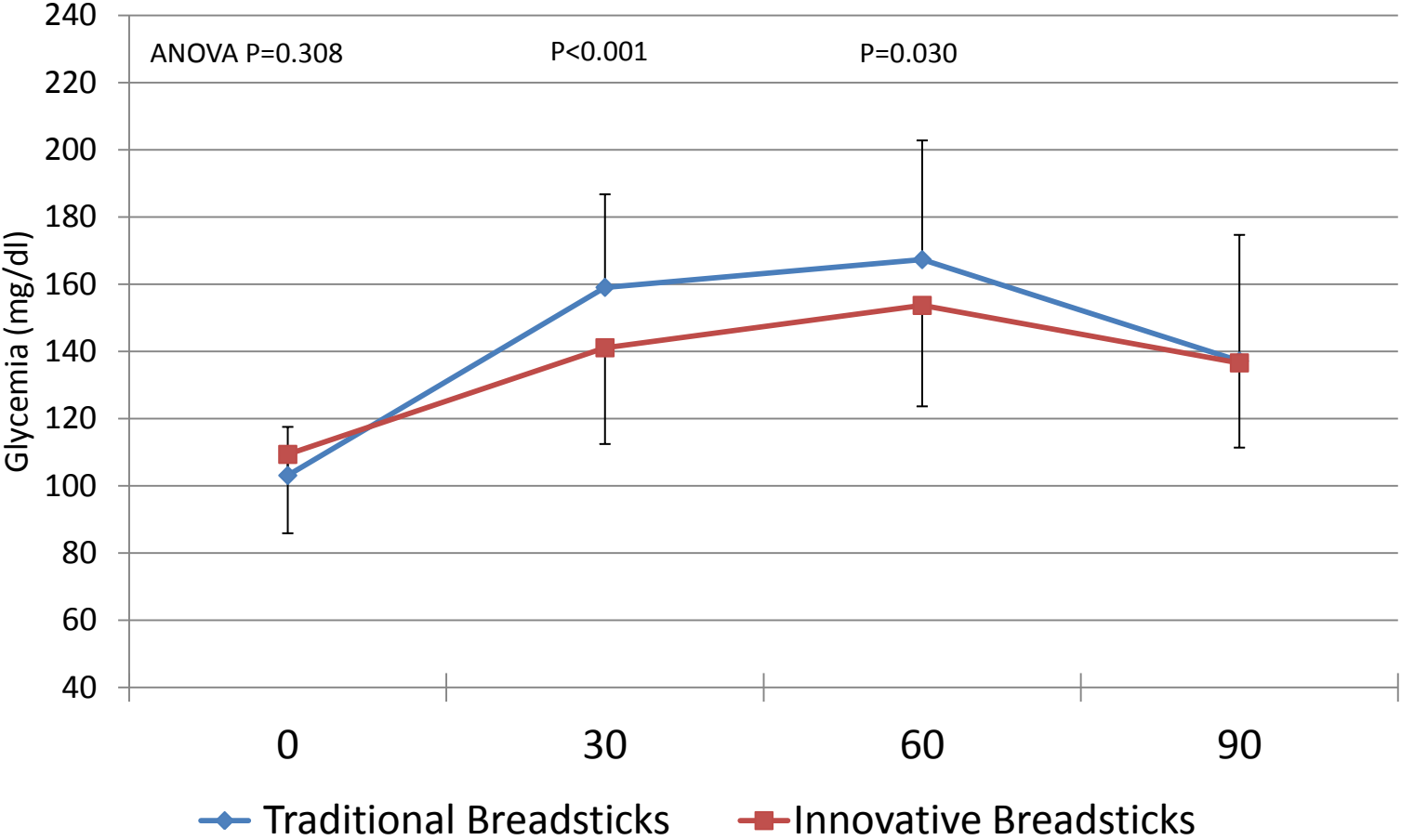
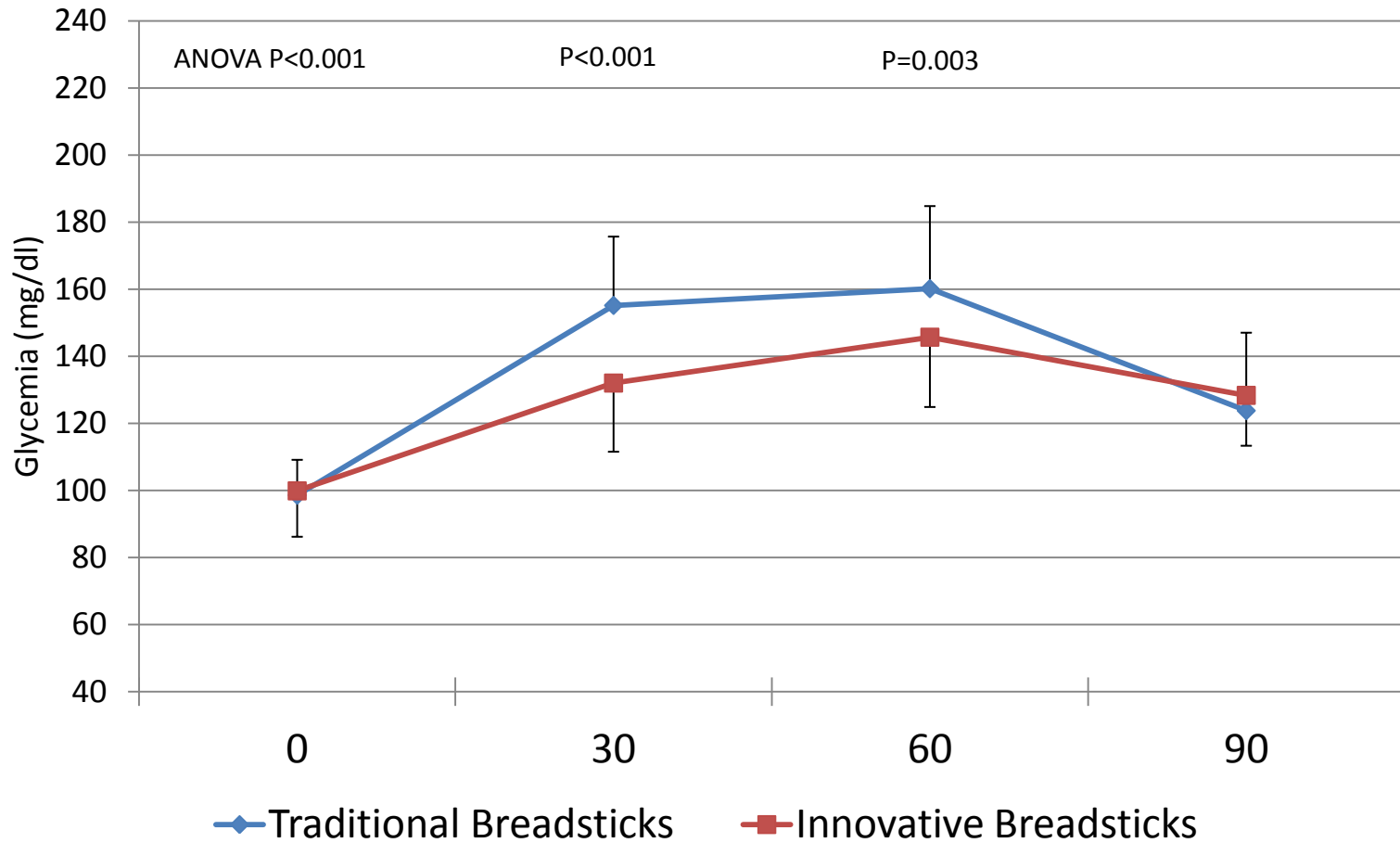


Fig. 15 - Glycemic curve after a breadsticks-based meal
All subjects (n = 27)



**Fig. 16 - Glycemic curve after a breadsticks-based meal
Subjects without DM (n = 21)**



**Fig. 17 - Glycemic curve after a breadstick-based meal
Subjects with DM (n = 6)**

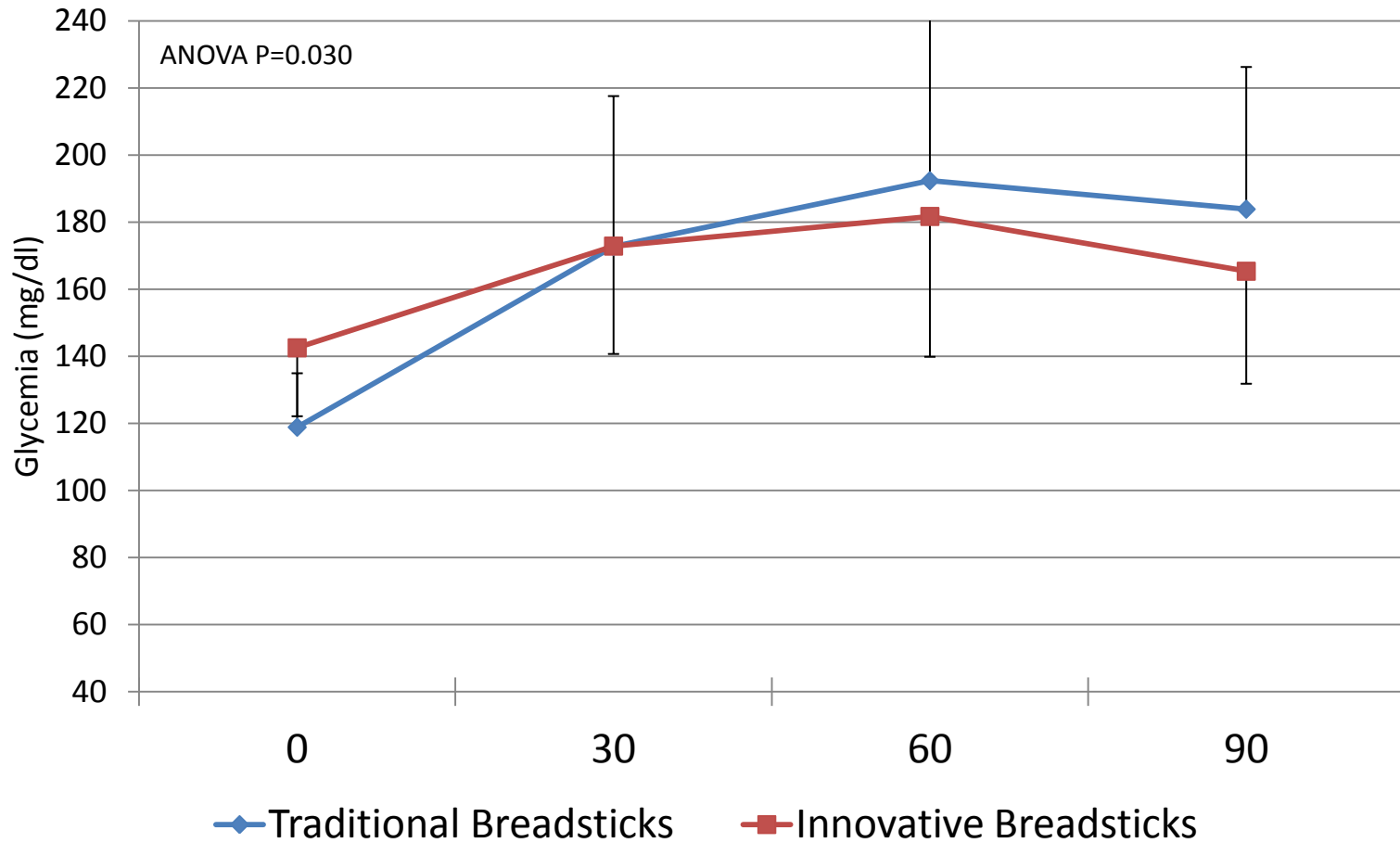


Fig. 18 - Glycemic peak after a breadsticks-based meal

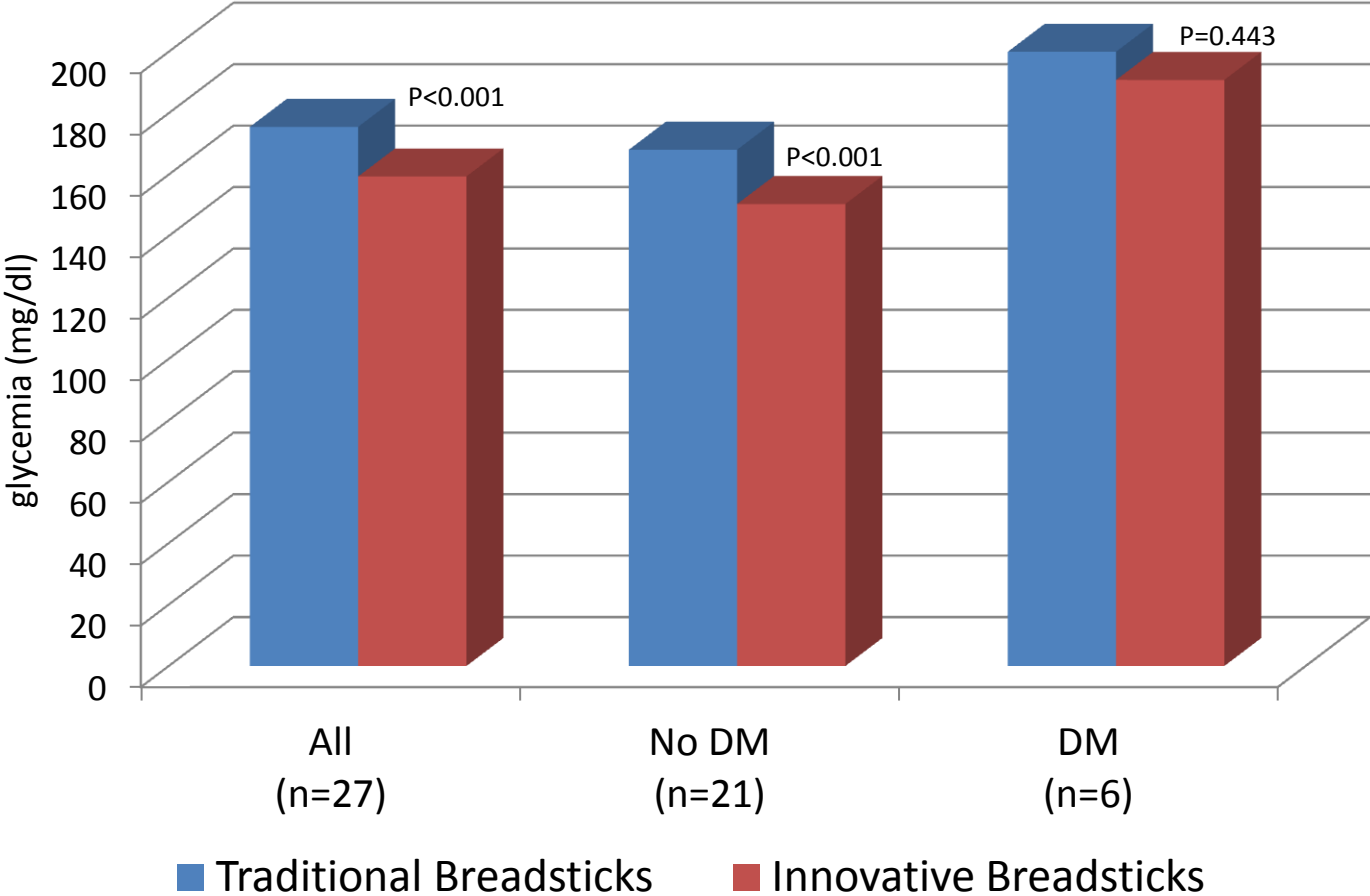


Fig. 19 - Blood sugar increase vs. baseline after a breadsticks-based meal

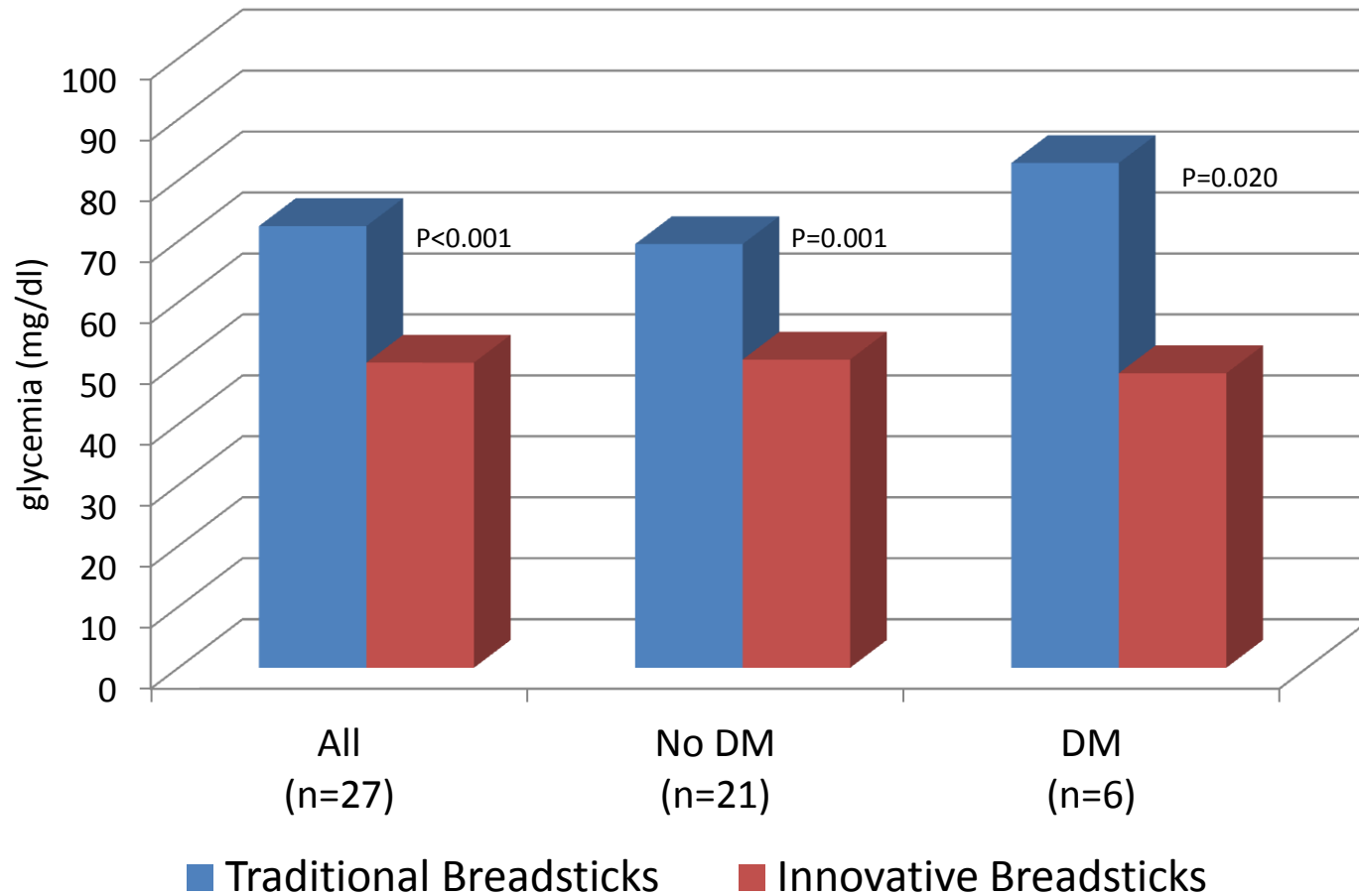


Fig. 20 - Area under the glycemic curve after a breadsticks-based meal

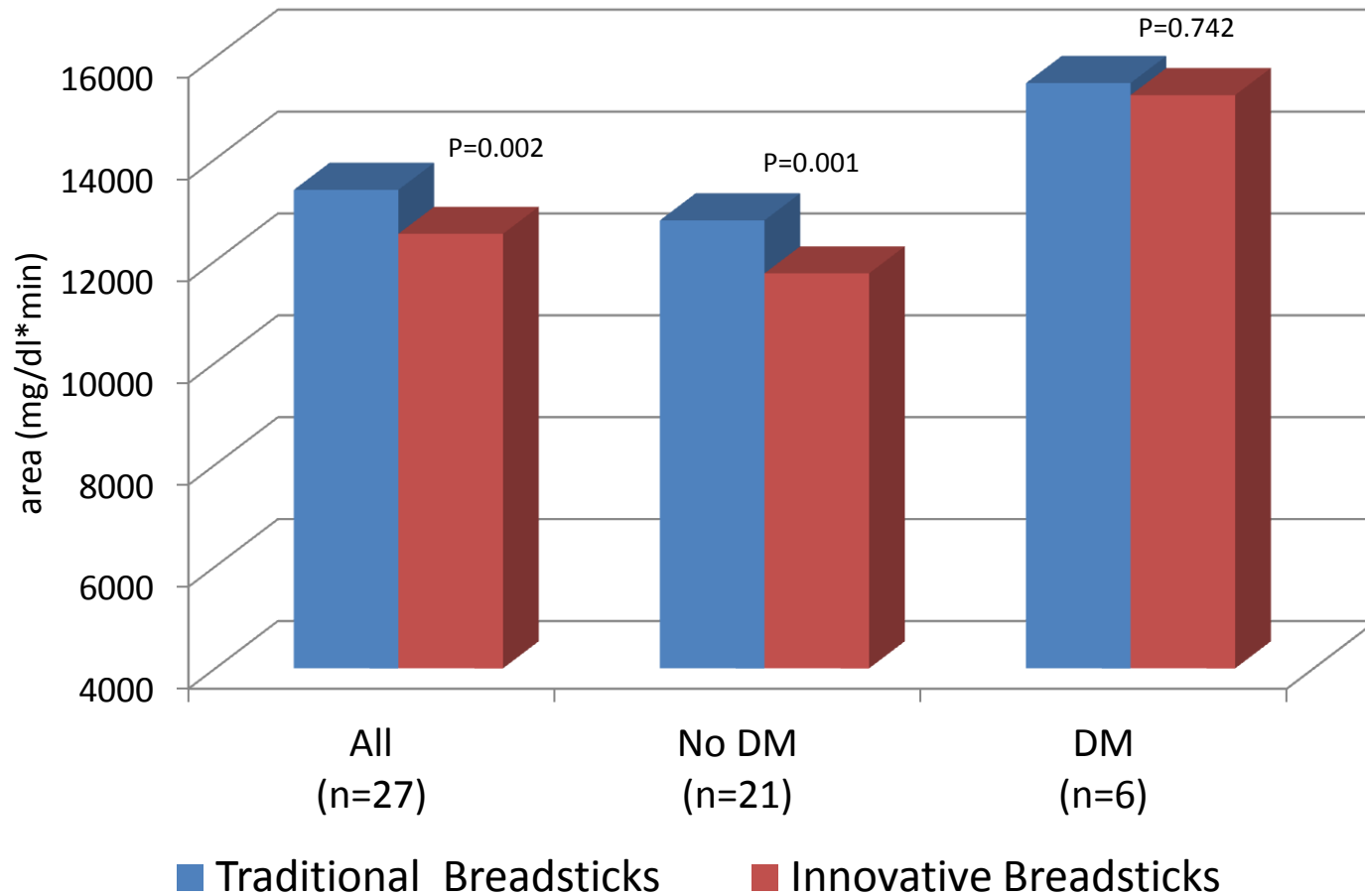


Fig. 21 - Incremental glycemic area after a breadsticks-based meal

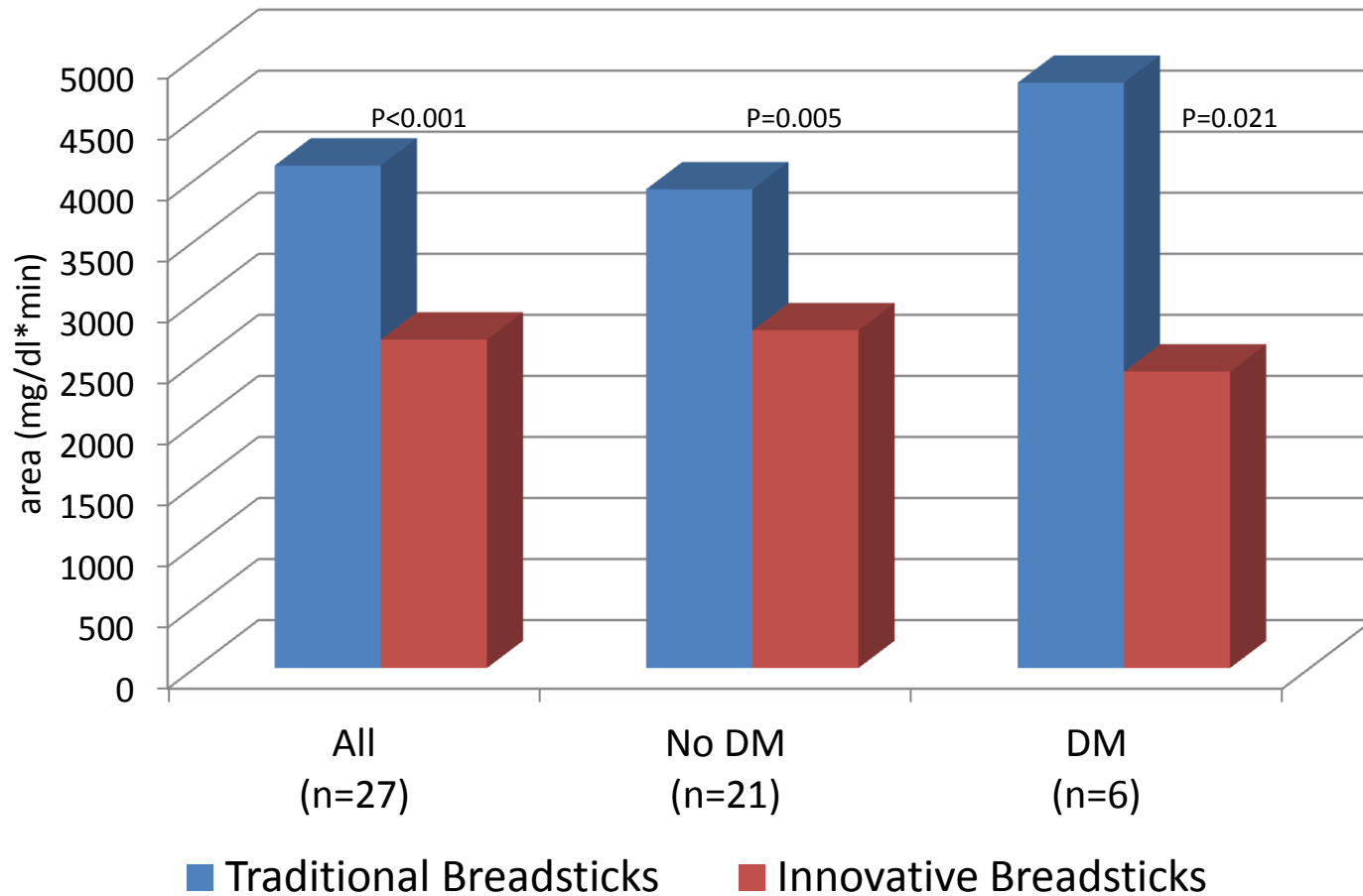
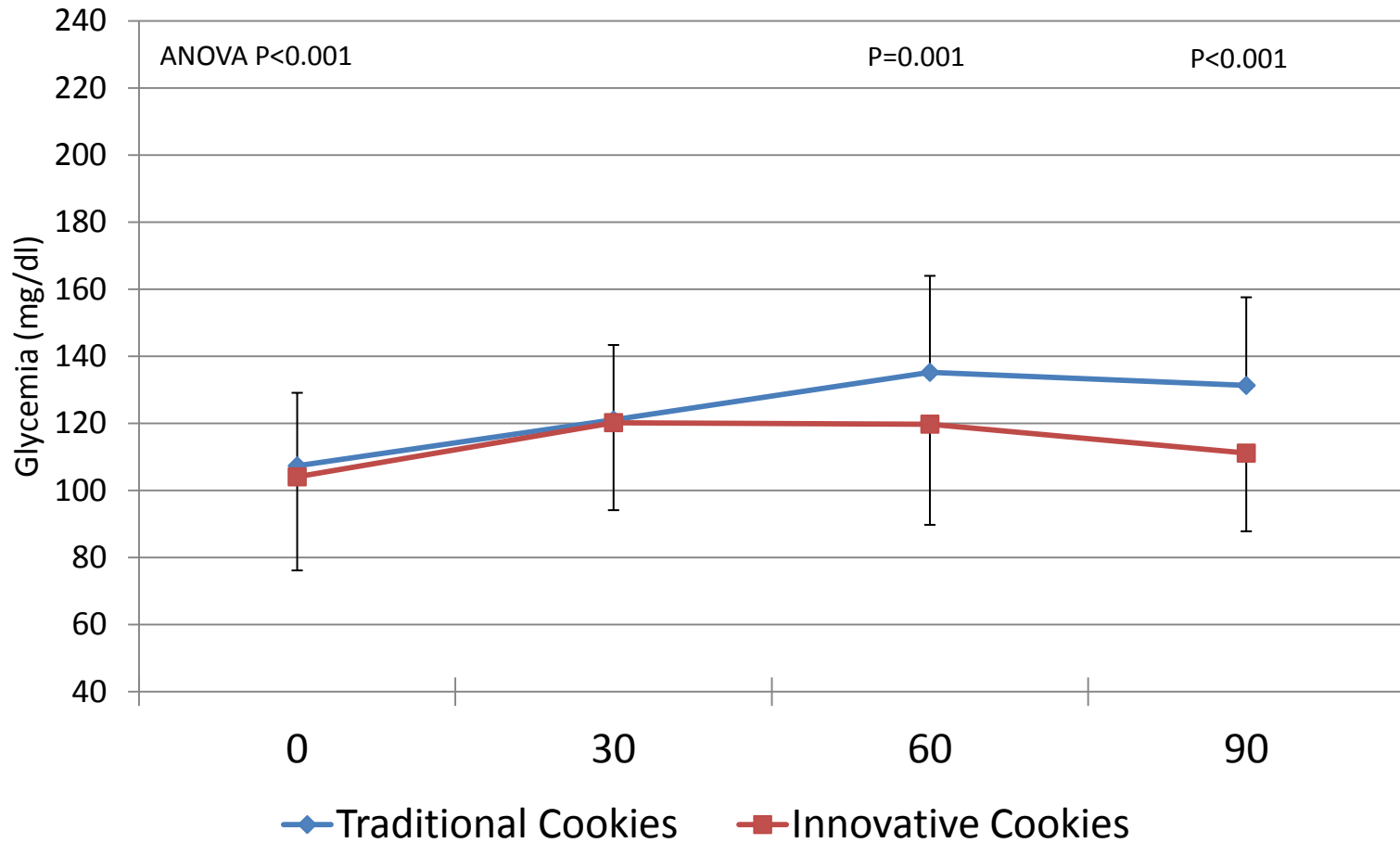
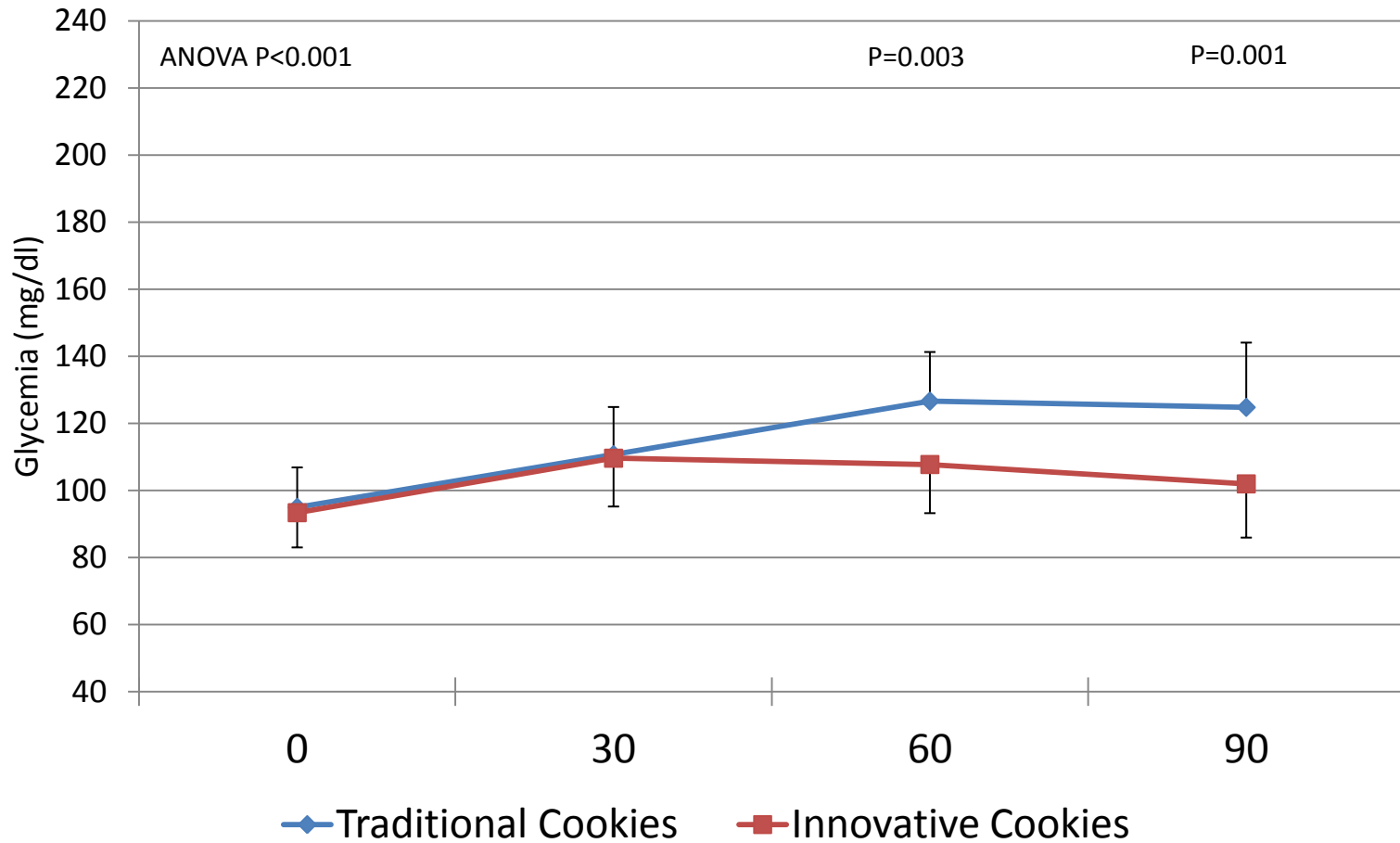


Fig. 22 - Glycemic curve after a biscuits-based meal
All subjects (n = 27)



**Fig. 23 - Glycemic curve after a biscuits-based meal
Subjects without DM (n = 17)**



**Fig. 24 - Glycemic curve after a biscuits-based meal
Subjects with DM (n = 10)**

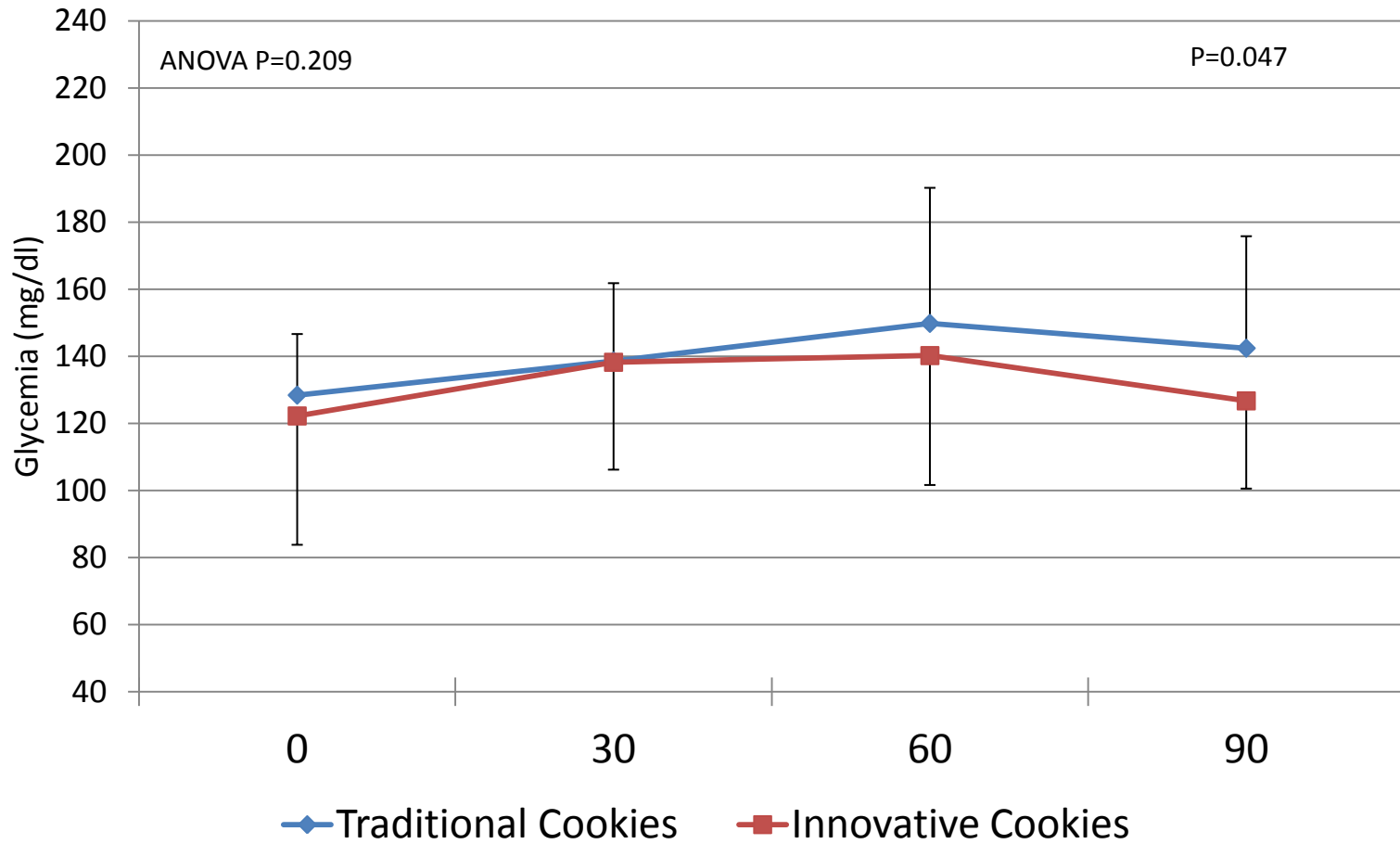


Fig. 25 - Glycemic peak after a biscuits-based meal

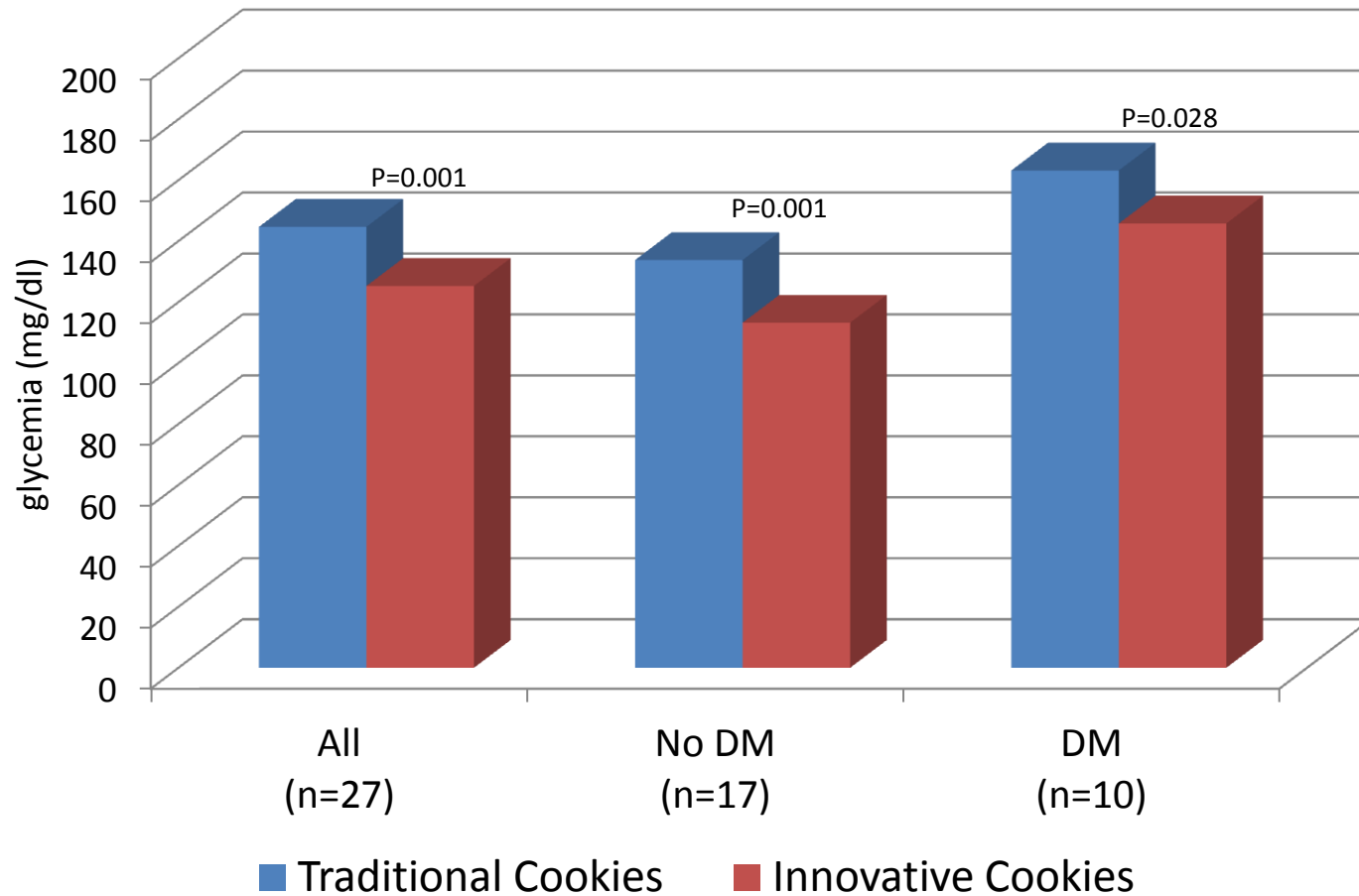


Fig. 26 - Blood glucose increase vs. baseline after a biscuits-based meal

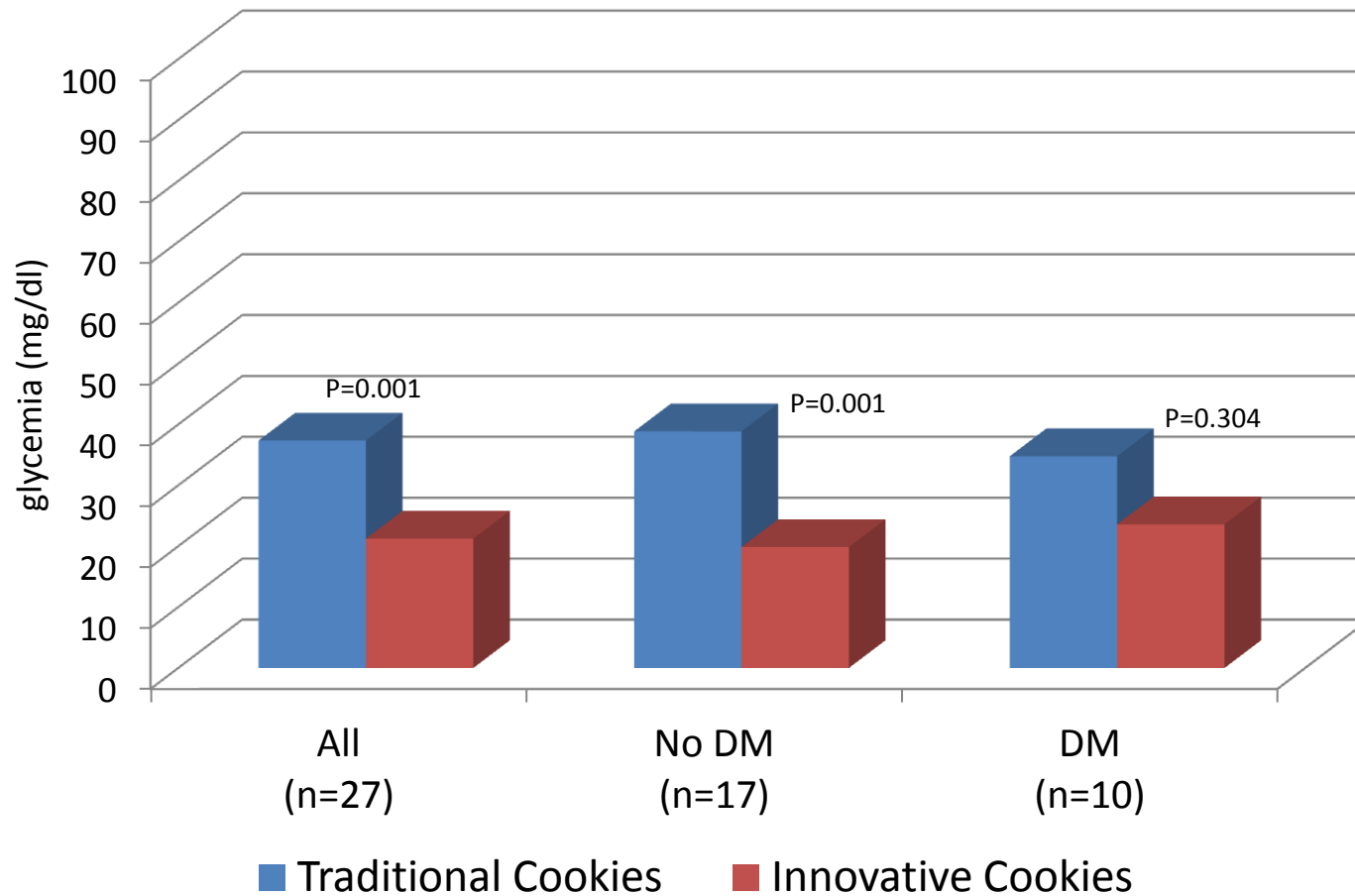


Fig. 27 - Area under the glycemic curve after a biscuits-based meal

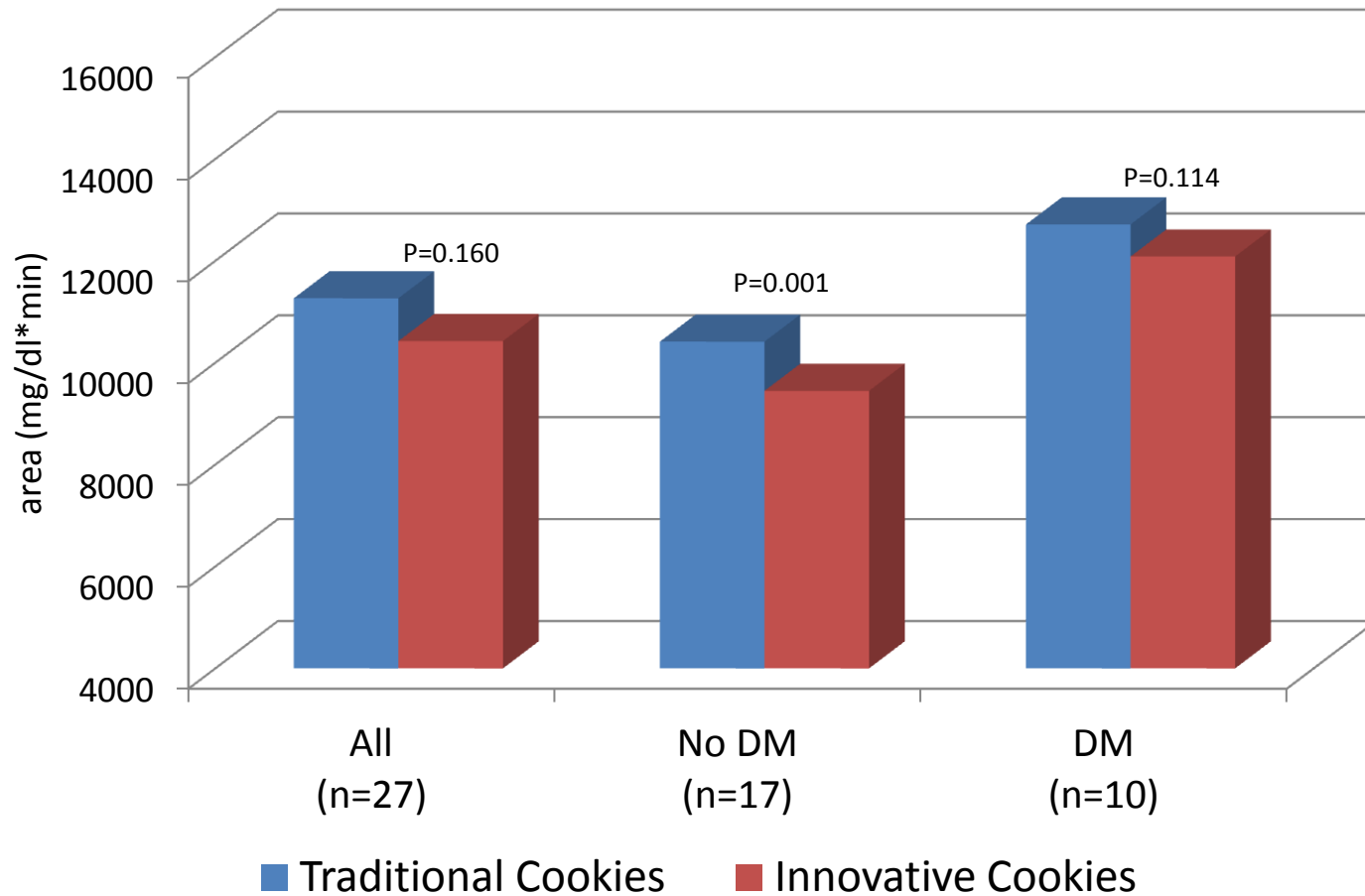


Fig. 28 - Incremental glycemic area after biscuits-based meal

