Pork Consumption and serum irisin levels in type 2 diabetes

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Co-operative Research Centre for Integrative Pork

By

Prof Manohar L Garg
Dr Rachel Wong
Prof Peter RC Howe

Nutraceuticals Research Group
University of Newcastle
Callaghan, NSW 2308
AUSTRALIA

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Executive Summary

Irisin, a myokine encoded by FNDC5 gene, expressed and produced by human muscle and adipose tissue, has been reported to stimulate conversion of white adipose tissue to brown adipose tissue and increase the expression of uncoupling protein 1. Health beneficial effects of exercise are thought to be mediated via increased production of irisin. Since the discovery of irisin, a large volume of literature relating its physiological effects, browning effect and metabolic function (Bostrom et al 2014; Hecksteden et al 2013; Huh et al 2012) has been published. Administration of irisin to mice through adenoviral delivery system exhibited browning of white adipose tissue at specific points associated with modest but significant weight loss (Bostrom et al 2012). Lower levels of circulatory irisin have been reported in type 2 diabetics and are positively correlated with age, BMI, plasma cholesterol and blood pressure in non-diabetics (Hojlund et al 2013; Liu et al 2013).

Pork is the most widely consumed meat in the world and serves as an excellent source of dietary protein as well as containing other vitamins and minerals. However, pork has been perceived as a fatty meat and therefore believed to be less healthy compared to other meats; this may have contributed to the lower rates of consumption in Australia. The amount of protein in a serving of pork varies according to the leanness of the cut. Regular consumption of lean pork has been shown to produce improvements in plasma lipid profiles similar to consumption of lean red meat (veal) (Rubio et al 2006). In fact, there is growing evidence that regular consumption of lean pork as a protein source in conjunction with regular exercise can improve cardio-metabolic health outcomes in diabetic and overweight patients (Wycherley et al 2010; Murphy et al 2012).

Increased lean muscle mass, as in the case of athletes, has been shown to be associated with increased blood irisin levels. Diet has a significant effect on muscle mass and type 2 diabetes development and glycaemic status. Pork is a rich source of protein and zinc which helps the body produce the muscle building hormone, testosterone. The aim of this project was to determine if regular consumption of pork meat providing high quality protein will improve muscle mass and increase circulating irisin levels in people with established type 2 diabetes.

This was a nutrition intervention study, where people with type 2 diabetes were assigned to consume 5 serves or 750g (women) or 7 serves or 1000g (men) of fresh unprocessed pork weekly and asked to incorporate it into their habitual diet for a 4-week period. Blood was collected at baseline and again on their post-intervention visit for analysis of blood lipid levels (plasma cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride) and glycaemic indices (fasting blood glucose, insulin, insulin resistance as determined by HOMA-IR). On each occasion, prior dietary intakes and physical activity were also assessed.

Results show that there were no differences in physical activity, energy or macronutrient intakes during the study period. Statistical analyses using paired, two-tailed t-test showed no effect of pork consumption on plasma irisin levels. All the glycemic indices and blood lipid levels also remained unchanged following dietary intervention with pork meat. Energy intake was significantly increased by inclusion of dietary pork. Pork consumption resulted in a significant reduction of percent body fat and a concomitant increase in muscle mass.

In conclusion, pork consumption for a short (4 weeks) period of time increased muscle mass and reduced percent body fat; however, these changes in body composition were not followed by a parallel increase in plasma irisin levels or glycaemic indices. The current findings suggest that regular consumption of a pork-enriched high protein diet, despite its failure to stimulate irisin production, may be included in the diets of people with type 2 diabetes.

Introduction

Type 2 diabetes is a metabolic disorder that is characterized by hyperglycemia (high blood sugar). Its development is a result of lifestyle and genetic factors and, if uncontrolled, can result in a number of severe health complications. Obesity and insulin resistance are commonly associated with the development of type 2 diabetes.

Adipose tissue is predominantly composed of two tissue types, white adipose tissue (WAT) and brown adipose tissue (BAT). White adipose tissue is the primary site of energy storage and is responsible for the release of adipokines. Adipokines are reported to induce insulin resistance and metabolic syndrome. Brown adipose tissue on the other hand is associated with an increase in heat production, total energy expenditure, reduced obesity and decreased insulin resistance.

Irisin is a novel muscle hormone (myokine) that drives the conversion of white adipose to brown adipose tissue (Novelle et al 2013). Recent studies have indicated that irisin levels are decreased and are inversely associated with newly diagnosed diabetes, suggesting that irisin may play an important role in glucose intolerance and the development of diabetes mellitus (Choi et al 2013).

Transgenic mice that over-express muscle PGC1-α resist age-related obesity and diabetes and have an extended life-span (Benz et al 2009). A recent study by Bostrom et al (2012) reported that expression of the exercise- and PGC-1α induced irisin drives brown fat-like development of white fat and thermogenesis. However, few studies have examined the relationship between irisin levels and glucose tolerance in subjects with normal glucose tolerance (NGT) or type 2 diabetes. Increased lean muscle mass, as in the case of athletes, has been shown to be associated with increased blood irisin levels (Pardo et al 2014). Dietary protein manipulation is known to have a significant effect on muscle mass and type 2 diabetes development and status.

For the first time, this study explored the potential for consumption of pork, as a source of protein, to enhance irisin production and favourably influence biomarkers of insulin resistance in adults with type 2 diabetes.

Methodology

SUBJECTS

Male and female participants with type 2 diabetes aged between 18 and 75 years old who were regular consumers of meats other than pork were recruited through placement of recruitment flyers on noticeboards at the University of Newcastle, local medical and pathology centers, pharmacies and diabetes education centers and through the Hunter Medical Research Institute volunteer register to participate the trial. Subjects with gestational diabetes, insulin dependence, active hepatitis/liver cirrhosis, chronic renal failure on hemodialysis, congestive heart failure or other known major disease were precluded from the study, as well as current smokers and pregnant or breast feeding women. Individuals who consumed more than one serve of pork per week were also excluded.

All procedures involving human subjects were approved by the Human Research Ethics Committee at the University of Newcastle, Callaghan, Australia and the trial was registered with the Australia Clinical Trial Registry (ACTRN12614000840684). Written informed consent was obtained from all subjects prior to their participation in the study.

STUDY DESIGN

Of a total of 71 volunteers who were screened for eligibility, 26 were recruited to commence the dietary intervention with supplied pork incorporated into their diet. A total of 26 omnivorous men and women with low habitual pork consumption (<1 serve per week) were recruited for the intervention trial. The volunteers were supplied with 5 serves (women) or 7 serves (men) of fresh unprocessed pork weekly and asked to incorporate (substituted their usual meats) it into their habitual diet for a 4-week period. Volunteers attended the research clinic on their first and last visit for an approximate of 1 hour in duration and the following assessments were made at each of these visits: weight and height (to calculate BMI (kg/m^2), body composition (assessed using the InBody bioelectrical impedance analysis), 3-day dietary recall, 3-day physical activity recall, irisin levels, blood glucose, insulin and lipid profile measurements.

DIETARY INTERVENTION

Participants were supplied with fresh unprocessed pork and asked to incorporate it into their habitual diet for a 4-week period. Based on our previous trials, females were required to consume 750g of pork weekly; males were required to consume 1000g of pork weekly. All volunteers visited the clinic at baseline to collect a selection of frozen meat products including lean pork steak, strips, diced, sausages and mince prepared from the same cut of meat i.e. lean leg portion. Participants were asked to complete a weekly log of pork consumption to record their daily consumption of provided meat for compliance assessment.

All volunteers were asked to keep a weekly log of pork consumption. Compliance to the dietary intervention was assessed at baseline and 4 weeks using meat consumption logs where subjects recorded their daily consumption of provided meat and meat intake. Participants were asked to maintain their normal physical activity throughout the study which was assessed at the same time- points as dietary intake using 3-day physical activity diaries.

OUTCOMES MEASURES

Irisin ELISA Assays

The primary outcome measure was the change in irisin concentration in plasma from venous blood collected from the participants at baseline and post dietary intervention. Irisin levels were analysed by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Adipogen, Liestal, Switzerland) with an intra-assay and inter-assay coefficient of variation <

10% and a sensitivity of 1ng/ml. Secondary outcome measures included the change in baseline levels of blood glucose, insulin, HOMA scores and lipid profile following the 4-week intervention.

Dietary intake

Volunteers were asked to record their dietary intake in a semi-quantitative 3-day weighed food record at baseline and again in the 4th week of the intervention period. Nutrient intakes were calculated using a computerized database (Foodworks 8, Xyris Software, Kenmore Hills, Australia) to ensure background diet was not a confounding factor to influence the test results.

Blood lipid profile

Volunteers were required at both baseline and final visits to have their blood collected via venipuncture from the antecubital vein using a vacutainer system. Blood was collected into a 9ml EDTA tube, 4mL lithium heparin tube and 2ml sodium fluoride tube (Becton Dickinson Bioscience, Ltd NSW, AUS) at baseline and again on their post-intervention visit for analysis of blood lipid levels. The EDTA tube samples were centrifuged at 3000 x g for 10 min (Heraeus Biofuge Stratos, Radiometer Pacific, AUS) to obtain plasma. The samples were analysed for fasting blood glucose, insulin, insulin resistance (HOMA), plasma cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride levels by Hunter New England Area Health Pathology Services (NSW, Australia) using standard automated analytical techniques.

STATISTICAL ANALYSIS

Data of subjects who completed the trial were analysed by paired 2-tailed t-test with a factor of time (baseline and post-intervention) to test for significant differences. The statistical package was SPSS 21 (IBM Software, Chicago, USA) and significance was set at $P<0.05$ unless otherwise stated.

RESULTS

Subject characteristics

Of the 26 subjects who were enrolled in the study, 2 withdrew prior to commencement of the study and 1 withdrew after commencement (Figure 1). Of those participants, 1 participant withdrew due to a personal illness and 2 withdrew as they could no longer commit to the study. A total of 23 subjects completed the intervention study. Characteristics of volunteers are presented in Table 1. The average (mean $\pm$ SD) age of the study cohort was 67.8±6.4 years and they were obese (BMI 32.4±5.2kg/m^2). Pork diet was well tolerated and participants remained healthy in general throughout the study period. Body weight and BMI remained unchanged following pork diet intervention (Table 1).

Dietary intakes

Energy intake (kJ) was significantly higher after 4 weeks of dietary intervention (8696±2543 kJ/day) compared to baseline (7525±2072kJ/day). Total fat and protein intakes remained unchanged following consumption of pork rich diet for 4 weeks, however, carbohydrate intake was significantly increased post-intervention (Table 3). This was due to most, but not all participants increasing their intake of rice, pasta, breads and potatoes during the pork intervention period, although no particular pattern of carbohydrate rich foods was observed. Amongst the various classes of fatty acids, intake of saturated fatty acids was elevated by inclusion of pork meat in the diet. Other fatty acid classes (monounsaturated or polyunsaturated) also increased after intervention, however, the difference failed to reach significance. Micronutrients including thiamin, riboflavin and folate were also significantly higher following pork consumption. Moreover potassium intake was also significantly elevated

from 3.1±0.8g/day to 3.6±1.1g/day (P=0.004). Intake of other micronutrients (vitamins, minerals and trace elements) remained unchanged following pork consumption for a period of 4 weeks (Table 3). Alcohol intake was also similar before and after dietary pork intervention.

Physical Activity
Levels of low, moderate and vigorous activities remained unaltered following consumption of the pork rich diets (Table 4).

Body composition
Percent body fat of the study cohort was in excess of the normal range at baseline (>33.0%). Consumption of pork enriched diet for 4 weeks resulted in a significant reduction of percent body fat (32.4±7.0) with a concomitant increase of muscle mass (from 35.9±7.5kg at baseline to 36.5±7.6kg post-intervention, P=0.05) (Table 1).

Blood lipids levels and glycemic indices
Baseline plasma cholesterol (4.32±1.2mmol/L), LDL-cholesterol (2.55±0.9mmol/L), HDL-cholesterol (1.17±0.3mmol/L) and triglyceride (1.60±0.7mmol/L) levels were all within the normal range. Following consumption of pork enriched diet for 4 weeks, the blood lipid levels remained unaffected (Table 2 and Figure 2). Glycemic indices including fasting blood glucose (8.3±2.4mmol/L), insulin (11.0±7.8mIU/L) and HOMA-IR (4.13±3.3) at baseline confirmed the diabetic status of the study participants. Administration of pork rich diet for a period of four week had no effects on any of the glycemic indices measured (Table 2 and Figure 3).

Plasma Irisin levels
Circulating irisin levels remained unaltered following consumption of the pork rich diets (Figure 4).

Discussion

This is the first study to investigate effects of regular consumption of pork on circulating irisin levels. Lifestyle changes including physical activity, exercise and nutrition interventions have previously been shown to alter body composition i.e. a reduction in fat mass with a concomitant increase in lean muscle mass. Parallel to increase in muscle mass, exercise and/or physical activity has also been shown to increase expression of irisin, a myokine released from the muscle tissue. However, there are no reports in the literature on nutritional regulation of irisin. Therefore, in this study we investigated whether consumption of fresh pork meat, increases protein intake, muscle mass and consequently results in increased plasma irisin levels in people with established type 2 diabetes. Circulating irisin has been previously shown to respond rapidly following muscle building (endurance and strength training) exercise (Norheim et al 2014), therefore, 4 weeks of protein rich pork intervention was considered adequate to enhance muscle mass and irisin levels. Reduced irisin levels are observed in type 2 diabetics (Hojlund et al 2013; Liu et al 2013); therefore, dietary strategies to increase circulating irisin levels may prove to be beneficial for control of blood glucose levels due to the fact that irisin plays an important role in the conversion of white adipose tissue into brown adipose tissue. In this study, plasma irisin levels remained unaltered by the inclusion of pork meat in the regular diet of the people with type 2 diabetes.

Participants were instructed not to make any changes in their usual diet other than including the prescribed amount of pork meat, maintain their usual physical activity level and were asked to report if their medication regimen changed during the course of the study. Since all forms of pork meat products including steak, strips, diced, sausages and mince were prepared from the same cut (lean leg portion) of meat, the nutrient content of all forms per serve was the same. Participants were compliant to the pork intervention and no adverse events were reported. The results presented suggest that the participants did not modify their habitual physical activity level; however, as expected, their protein intake (104 g/day pre and 118 g/day post-intervention) increased upon inclusion of pork in the diet, although the difference did not reach significance. Perhaps increasing dietary proteins in the form of pork meat or other means alone is insufficient to increase irisin levels. Future studies should combining exercise/physical activity and nutrition interventions in efforts to increase circulating irisin levels for the benefit of the diabetic patients. Unexpectedly, carbohydrate intake was also significantly increased following pork intervention. This was due to most, but not all participants increasing their intake of rice, pasta, breads and potatoes during the pork intervention period, although no particular pattern of carbohydrate rich foods was observed.

Since the selection of meat products including lean pork steak, strips, diced, sausages and mince were all prepared from the same cut of meat i.e. lean leg portion, the nutrient composition or the cut preferences or their fat content were not a factor in determination of blood biomarkers of cardiometabolic risk factors. Despite the increase in saturated fat intake following inclusion of pork meat in the diet, blood lipid (total and LDL-cholesterol, HDL-cholesterol and triglyceride) levels and glycemic indices (fasting glucose, insulin, HOMA-IR) were unaltered, suggesting pork meat can be included in the daily diets of diabetes patients and may not further impact their glycemic control or increase their risk of developing cardiovascular disease. Importantly, as mentioned above, despite increased energy intake, BMI did not increase and there was a favourable change in body composition. This report adds to the growing body of evidence for the healthiness of pork. Howe et al (Pork CRC 3B-111) recently demonstrated that regular consumption of lean pork was equally healthy as beef or chicken showing no difference between the pork, beef or chicken diet for body weight, body mass index (BMI) or any other index of adiposity. However, the project did not include collection and analysis of blood samples for biomarkers of cardio-metabolic health.

It is noteworthy that irisin has only been recently (2012) discovered and very little is known about its regulation. A number of other factors including genetic makeup, age, gender, menopausal status, smoking, alcohol consumption, blood pressure, hormones obesity, hyperlipidemias and lipid-lowering drugs may influence irisin levels that have not been taken into consideration in this study. Also lack of a control arm remains a limitation of the study. In conclusion, we provide proof of concept that despite no change in irisin levels, regular consumption of pork does not worsen glycemic control or increase cardiovascular disease risk in people with type 2 diabetes.

Figure 1. Consort diagram.

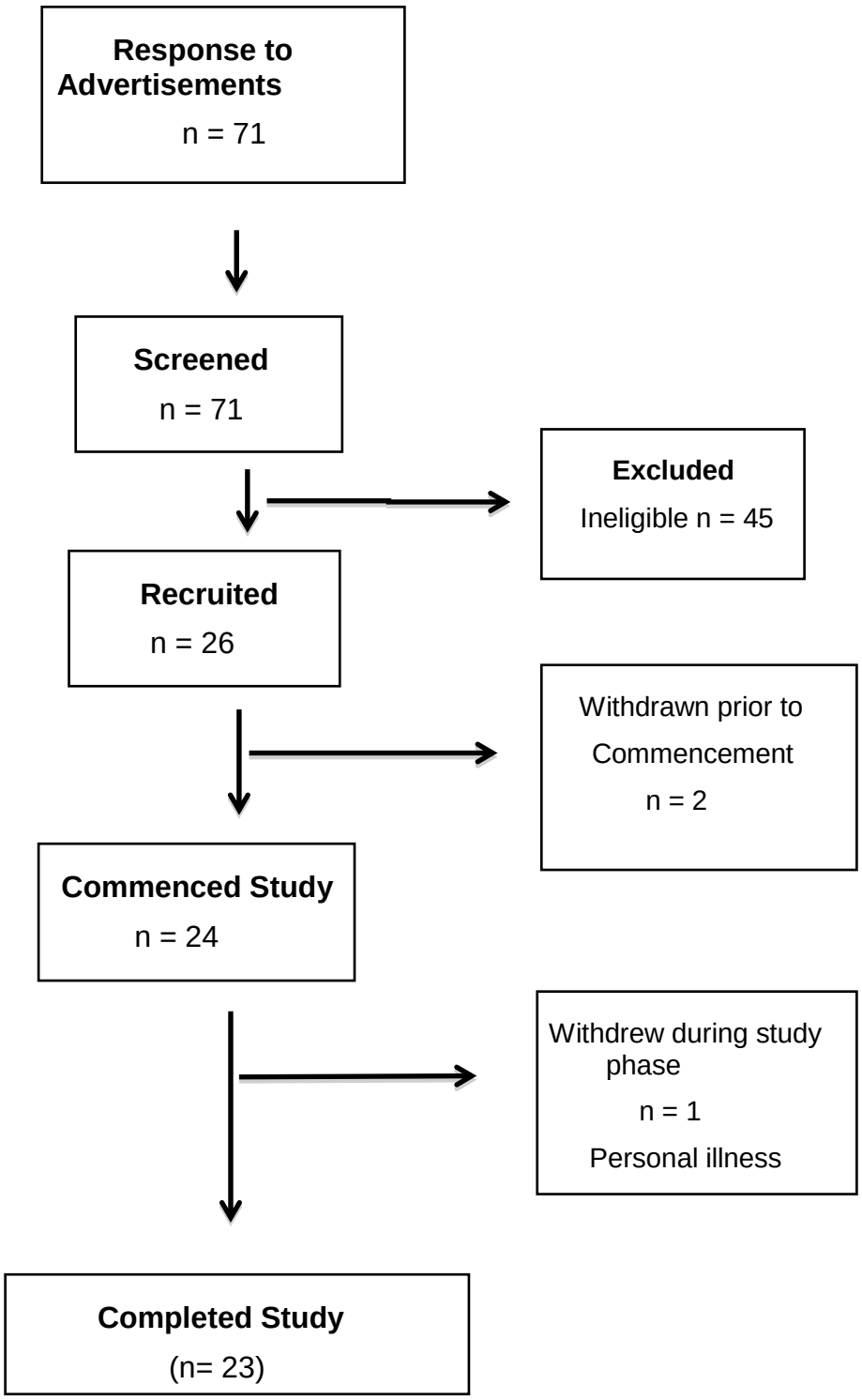

Table 1. Effects of pork intervention on anthropometric and body composition measurements in people with type 2 diabetes*.

	Baseline	Post-intervention	P value
	N=23	N=23	
Gender *n*	17M / 6W	17M / 6W	-
Age (yrs)	67.8±6.4	67.8±6.4	-
Weight (kg)	98.9±20.7	98.8±20.9	0.87
Height (cm)	175±9.1	175±9.1	-
BMI (kg/m^2)	32.4±5.2	32.3±5.2	0.59
Fat Mass (kg)	34.8±11.1	34.1±10.8	0.09
% Body Fat	33.5±7.0	32.4±7.0*	0.01
Muscle mass (kg)	35.9±7.5	36.5±7.6*	0.05

* Data presented as mean±SD.

Table 2. Effects of pork intervention on blood lipid profile and glycemic indices in people with type 2 diabetes*.

	Baseline	Post-intervention	P value
	N=23	N=23	
Glucose (mmol/L)	8.3±2.4	8.4±2.3	0.830
Insulin (mIU/L)	11.0±7.8	14.1±15.0	0.284
HOMA-IR	4.13±3.3	5.44±7.0	0.361
Total cholesterol (mmol/L)	4.32±1.2	4.43±1.1	0.623
Triglycerides (mmol/L)	1.60±0.7	1.59±0.7	0.902
LDL-cholesterol (mmol/L)	2.55±0.9	2.63±1.0	0.475
HDL-cholesterol (mmol/L)	1.17±0.3	1.17±0.4	0.866

* Data presented as mean±SD.

Table 3. Nutrient intakes of study participants prior to and following intervention with pork rich diet.

	Baseline	Post-intervention	P value
Energy (kJ)	**7525±2072**	**8696±2543***	**0.02**
Protein (g)	104±33	118±36	0.11
Fat (g)	63±28	76±34	0.07
-SFA (g)	**23.4±11.4**	**29.3±14.4***	**0.03**
-MUFA (g)	24.9±11.6	29.2±13.5	0.15
-PUFA (g)	9.3±4.7	10.6±4.8	0.14
- 18:2n-6	7.3±3.7	8.5±4.2	0.11
- 18:3n-3	1.2±0.6	1.2±0.5	0.61
- 20:5n-3	0.2±0.5	0.1±0.3	0.61
- 22:5n-3	0.1±0.2	0.1±0.2	0.81
- 22:6n-3	0.2±0.5	0.2±0.3	0.99
- TFA	1.0±0.5	1.4±1.5	0.22
CHOs (g)	**166±49**	**190±54***	**0.03**
-Fibre (g)	24.2±6.9	26.2±9.5	0.21
-Sugars (g)	67.2±27.8	81.2±31.6	0.10
-Starch (g)	98.0±30.4	107.9±41.6	0.20
Alcohol (g)	13.6±27.8	15.2±24.9	0.50
Cholesterol (mg)	358±139	456±539	0.41
Thiamin (mg)	**2.0±1.1**	**2.7±1.0***	**0.01**
Riboflavin (mg)	**1.7±0.6**	**2.0±0.6***	**0.04**
Niacin (mg)	27.7±13.0	31.9±12.8	0.06
Folate (mg)	**496±156**	**554±146***	**0.03**
Vitamin B12 (μg)	4.7±2.7	4.9±3.2	0.87
Vitamin E (g)	9.0±5.0	8.2±2.8	0.44
Sodium (mg)	2310±795	2518±1388	0.45
Potassium (mg)	**3114±803**	**3620±1095***	**0.004**
Vitamin C (mg)	94±40	120±69	0.08
Magnesium (mg)	328±91	362±116	0.07
Calcium (mg)	693±258	776±223	0.06
Phosphorus (mg)	1543±388	1718±403	0.07
Iron (mg)	11.5±2.7	12.4±3.5	0.23
Zinc (mg)	13.2±6.8	14.2±5.8	0.60
Selenium (μg)	102±45	107±36	0.69
Iodine (μg)	171±62	166±44	0.70

*Data presented as mean±SD.

Table 4. Effects of pork intervention on low, moderate and vigorous physical activity in people with type 2 diabetes*.

	Baseline (MET-min/wk)	Post-intervention (MET-min/wk)	Significance
Low Physical Activity[1]	198±280 (n=2)	396±0 (n=1)	
Moderate Physical Activity[2]	1698±650 (n=13)	1867±838 (n=13)	0.617
Vigorous Physical Activity[3]	4283±2015 (n=8)	4807±1277 (n=9)	0.656
Total Physical Activity	2467±1883 (n=23)	2953±1838 (n=23)	0.108

MET, metabolic equivalent time: *Data presented as mean±SD.
[1]Low Physical Activity defined as <600 MET-min/week
[2]Moderate Physical Activity defined as 600-3000 MET-min/week
[3]Vigorous Physical Activity defined as >3000 MET-min/week

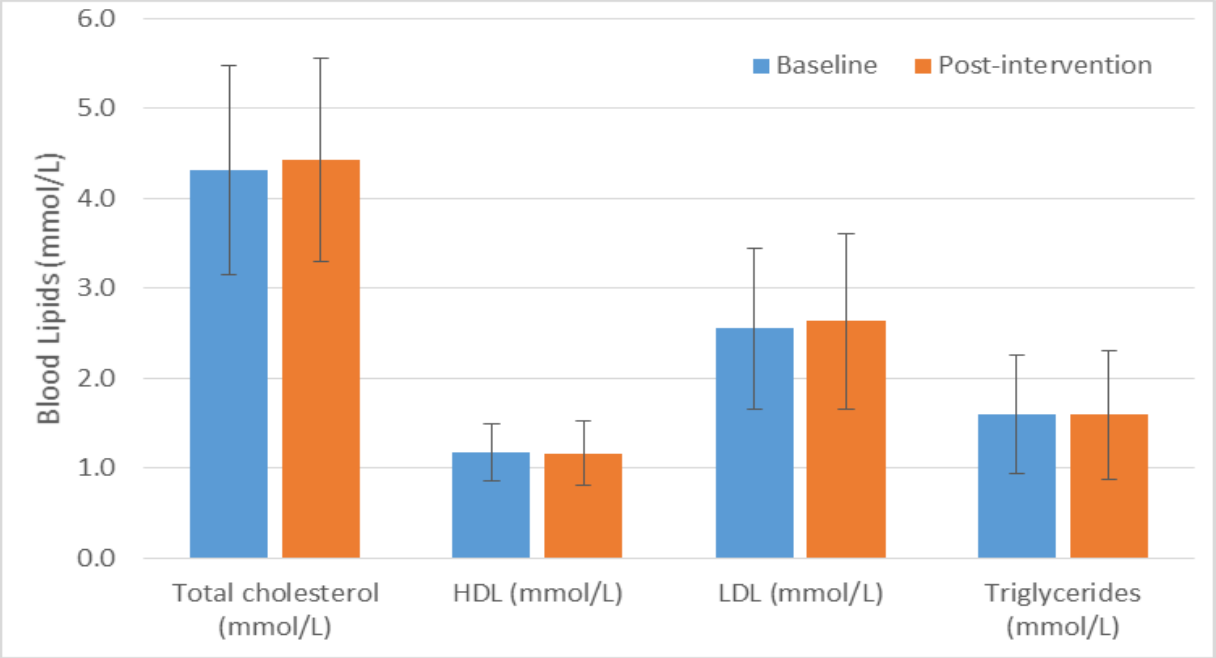

Figure 2. Blood lipid levels at baseline and post-intervention. Data presented as mean±SD.

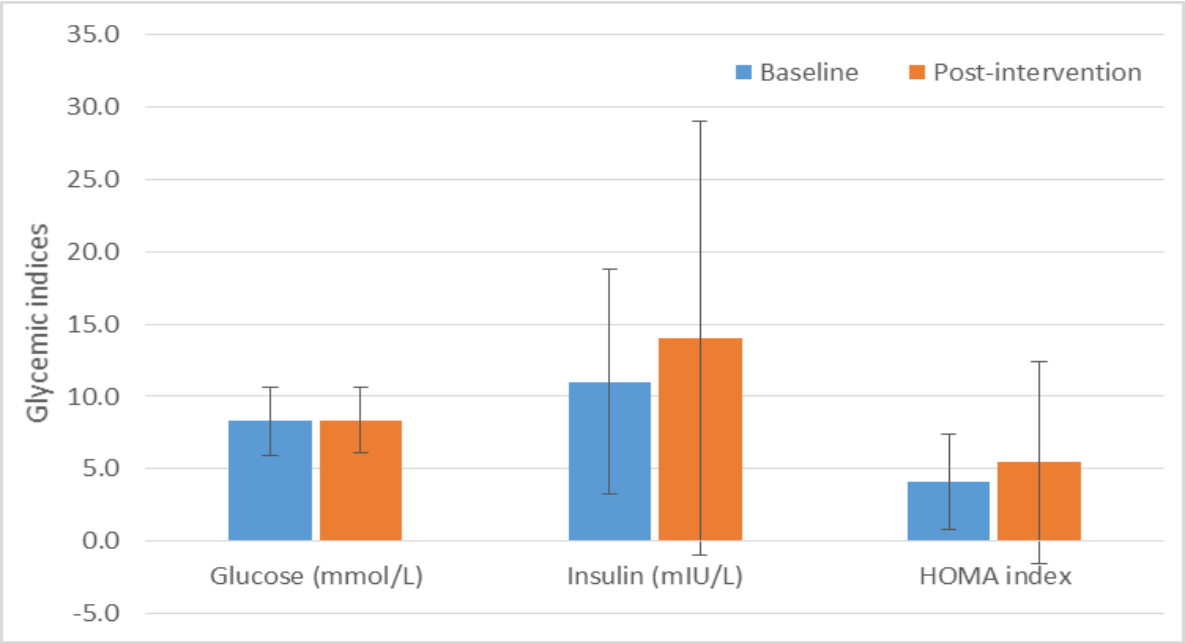

Figure 3. Glycemic indices at baseline and post-intervention. Data presented as mean±SD.

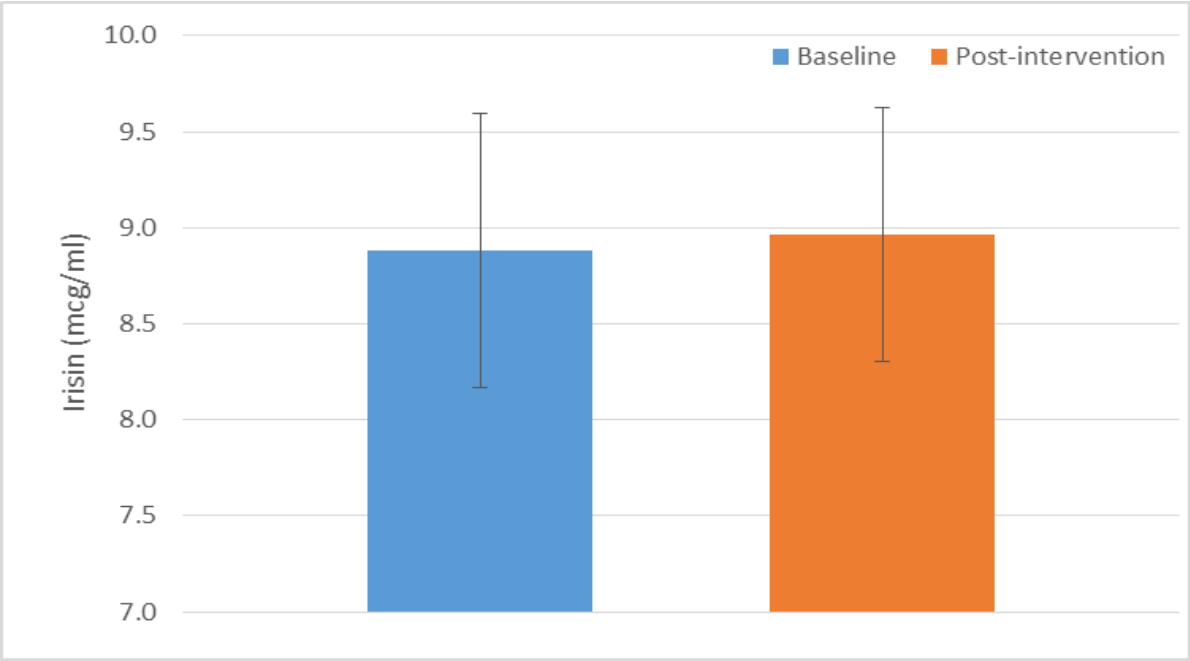

Figure 4. Plasma irisin levels at baseline and post-intervention. Data presented as mean±SD.

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