Received: 9 August 2018 | Revised: 29 December 2018 | Accepted: 22 January 2019

DOI: 10.1111/asj.13193

ORIGINAL ARTICLE

WILEY Animal Science Journal

Effect of broth from meat of linseed-fed cattle on glucose-stimulated insulin release in healthy male volunteers

Zoltan Bacs[1] | Katalin Szabo[2] | Levente Czegledi[3] | Jozsef Nemeth[2] | Peter Fraknoi[3] | Peter Kovacs[4] | Andras Javor[3] | Balazs Varga[2] | Bela Juhasz[2] | Zoltan Szilvassy[2]

[1]Faculty of Economics and Business Administration, University of Debrecen, Debrecen, Hungary

[2]Faculty of Medicine, Department of Pharmacology and Pharmacotherapy, University of Debrecen, Debrecen, Hungary

[3]Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen, Debrecen, Hungary

[4]Faculty of Medicine, Institute of Internal Medicine Building A, University of Debrecen, Debrecen, Hungary

Correspondence
Bela Juhasz, Faculty of Medicine, Department of Pharmacology and Pharmacotherapy, University of Debrecen, Debrecen, Hungary.
Email: juhasz.bela@pharm.unideb.hu

Abstract

Polyunsaturated fatty acid consumption has been shown to improve insulin sensitivity. We studied if administration of broth with beef meat enriched with polyunsaturated fatty acids influenced glucose-stimulated insulin release in healthy male volunteers. Broth was made either from cattles undergone dietary supplementation with lightly bruised whole linseed in addition to feeding ad libitum on grass silage (test meal) or from those fed grass silage alone (control meal). Oral glucose tolerance tests (OGTT) were performed in patients after a 6-day period of eating 300 ml broth containing 100 g meat once a day in addition to their otherwise normal mixed nourishment. During OGTT, blood samples were taken for blood glucose level and plasma insulin immunoreactivity before and 15, 30, 60, 90, 120, and 180 min after the glucose load. Glucose-stimulated maximum increase in plasma insulin immunoreactivity was 42 ± 6.6 and 81 ± 7.4 mU/ml ($p < 0.05$) after the test and the control meals, respectively. However, both fasting and postload blood glucose levels were the same after either meal period. The results suggest an insulin-sensitizing effect of food produced from beef cattle maintained on linseed diet in healthy human volunteers.

KEYWORDS
beef, fatty acid, flax, glucose, insulin

1 | INTRODUCTION

Eating a meal has long been realized to stimulate several short-term and long-term physiological adjustments. With the advent of the term "functional foods", it has been proposed that ingesting some foods may yield particular health advantages in terms of disease treatment and prevention beyond their nutritional property (Czegledi et al., 2011; Syngai et al., 2016; Ungvari et al., 2014).

Food with increased polyunsaturated fatty acid (PUFA) content has come into the limelight since the discovery that eskimo diet (very rich in fish oil) conferred protection on patients at risk of cardiovascular disease (Dyerberg, Bang, Stoffersen, Moncada, & Vane, 1978; Jump, Depner, & Tripathy, 2012). Protection from progression of atherosclerosis and the generation of cardiac arrhythmias provide the first line of evidence in this respect. Beyond direct cardiovascular protective effects, PUFA and PUFA-rich diet have recently been claimed to reduce the risk of type II diabetes possibly through a reduction of insulin resistance, the key metabolic abnormality that both precedes and characterizes type II diabetes (Cavaliere et al., 2016; Rudkowska, 2009). However, studies on the effect of

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2019 The Authors. *Animal Science Journal* published by John Wiley & Sons Australia, Ltd on behalf of Japanese Society of Animal Science

PUFA-rich food or PUFA-containing formulation on diabetes almost completely confine to those with fish oil. We therefore decided to investigate the effect of meat derived from steers maintained on lightly bruised linseed-rich forage on glucose-stimulated insulin release in healthy male volunteers. Here, we present in a pilot work that broth made from the meat of such animals inhibited glucose-stimulated insulin release in young males at normal blood glucose level. This suggests that beef meat rich in linseed-derived fatty acids may be of beneficial influence on insulin sensitivity in human.

2 | MATERIAL AND METHODS

2.1 | Animal feeding

Four Limousine steers were housed in a well-ventilated barn. Age, sex, and genetics were identical in all steers. Weight of the carcasses ranged from 280 to 300 kg. The animals were randomized, so as two animals served for control, whereas the other two steers were referred to as the "linseed" group. These animals were given "linseed forage", a lightly bruised whole linseed-rich forage concentrate that was formulated, so that total dietary oil intake was 8% of which approximately 10% was from the linseed milling product. Vitamin E was also added to the concentrate in the amount of 400 IU/kg (the same Vitamin E amount was given to the control animals as well). The animals within the same group were penned together and bedded on wood shavings.

At the beginning of the experiment, during a 14-day covariate period, all animals were fed on grass silage ad libitum and rolled barley was allocated individually to provide one-third of DM intake. The animals were then introduced to their "linseed" dietary treatment over a 7-day period. Feed levels were adjusted to grass silage ad libitum and the linseed milling product at a ratio of 60:40, forage concentrate on a DM basis. During the 14-day covariate period, feed intake was recorded and live weight measurements were made on five occasions. During the main experimental period, feed intake was recorded and weekly live weights were recorded. All weighings were done at the same time (13:30 hr) to reduce variations due to diurnal patterns of feed intake. Grass silage was fed at 14:00 hr daily, and the linseed/rich forage concentrate in two equal portions at 09:00 hr and 16:30 hr. The grass silage and the linseed concentrate were sampled three times per week for DM analysis in a forced-air oven at 105°C. Silage was offered at 110% of the previous consumption. Concentrates feed levels were adjusted weekly, based on the previous week's silage consumption. Samples of silage and concentrate were accumulated over 4-week periods and chemical composition assessed as described previously (Dewhurst et al., 1999). Following a period of 40-day diet, the animals were slaughtered. Animal experiment protocol number: OMFB-01009R2005.

2.2 | Lipid determinations from beef muscle

Complete cross-sections of the m. longissimus thoracis 10–12 rib level, 15 mm thick were freed of epimysium and adhering adipose tissue. The muscle was blended in a food processor and the lipids extracted from duplicate 10 g samples using chloroform:methanol (2:1, v/v;) (Folch, Lees, & Sloane Stanley, 1957). Neutral lipid and phospholipids were isolated from the lipid extract using silicic acid chromatography. Extracted lipid, 20–30 mg in approximately 1.0 ml chloroform was applied to a 500 mg silicic acid column (Isolute Si, Jones Chromatography, Hengoed, Glamorgan, UK) previously washed extensively with chloroform. The neutral lipid fraction was eluted with 10 ml chloroform and the phospholipid fraction (polar lipids) with 20 ml methanol. After the addition of the fatty acid standard, heneicosanoic acid methyl ester (Sigma, St. Louis, MO, USA), the solvents were removed under nitrogen and the lipids hydrolysed with 2 mol/L potassium hydroxide in methanol:water (1:1, v/v), containing 1 g/L hydroquinone as antioxidant, at 60°C for 1 hr. After dilution with water and removal of nonsaponifiables by three extractions with petroleum spirit (BP 40–50°C), the hydrolysate was acidified and the fatty acids extracted into petroleum spirit. After neutralization and drying, with solid sodium hydrogen carbonate and anhydrous sodium sulfate, the fatty acids were methylated with a solution of diazomethane in diethyl ether and their composition determined by gas-liquid chromatography. Samples were injected in the split mode, 70:1, onto a CP Sil 88, 50 m _ 0.25 mm fatty acid methyl esters (FAME) column (Chrompack UK Ltd, London, UK) with helium as the carrier gas. The output from the flame ionization detector was quantified using a computing integrator (Experimetria HU) and linearity of the system was tested using saturated (FAME4) and monounsaturated (FAME5) methyl ester quantitative standards (Thames Restek UK Ltd, Windsor, UK).

Fatty acid results are given as mg of fatty acid per 100 g wet tissue quantified by reference to the internal standard. Only the major fatty acids and minor components readily identified and relevant to the study are reported, representing over 90% of the total fatty acids present. The fatty acid reported as 16:1 cis consists of both the *n*−9 and *n*−7 isomers and contaminating branched seventeen-carbon fatty acids. The trans 18:1 isomers are incompletely resolved by this column and are reported as one value. In view of the complexity of the chromatogram in this area as a result of the wide range of 18:1 isomers in ruminant tissues (Hay & Morrison, 1973), some minor cross-contamination of the listed 18:1 isomers may also be present.

2.3 | Patient selection

Eighteen physically active, normocholesterolemic office employees (all male, mean age 25.2 ± 2.4 years) were studied. None of them had a history of hypertension, diabetes mellitus, or smoking. The mean body mass index was 23.6 ± 2.60 m^{-2}. Six of them were excluded from the study subsequent to an initial routine blood chemistry because of an increase in serum transaminase levels (three volunteers), an increased red cell sedimentation value (two volunteer), and low compliance regarding restrictions of physical activity during the investigation period (one volunteer). All subjects gave their informed consent for inclusion before they participated in the study. The study

was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of University of Debrecen, Hungary (license number: 22/08 SR).

2.4 | Study design

Oral glucose tolerance test ([OGTT] 75 g glucose in 200 ml of water) was performed in each patient twice, divided by a 2-month interval. The study was carried out and evaluated in a double blind fashion. The volunteers were randomized for receiving either broth got ready from meat of cattles maintained on linseed-rich diet (linseed broth) or that made from control cattles (control broth) once a day over a period of 6 days prior to the OGTT test (300 ml soup containing 100 g meat). The soup was prepared as follows: 100 g beef was cut into 2 × 2 × 2 cm cubes and put into 350 ml water (this included estimated loss) with 2 g salt, which was heated until boiling, than heat was reduced to simmer and maintained for 4 hr, after which the broth was removed from heat and let to cool. OGTT tests were preceded by an overnight fast and a 2-week period of restricted physical exercise and caffeine intake. The patients received the glucose solution at 08.00 hr. Venous blood samples were taken during fasting (immediately before glucose) and at 15, 30, 60, 90, 120, and 180 min after the glucose load. The samples were stored at −20°C and subsequently evaluated for plasma glucose level (mmol/L) and immunoreactive insulin responses (mU/ml). Plasma glucose was determined by means of an auto-analyzer (Beckman, Miami, FL, USA) using the glucose oxidase method. Immunoreactive insulin levels were assessed by radioimmunoassay method using antihuman antibody (IZINTA, Budapest, Hungary) as described previously (Kovacs et al., 2000).

2.5 | Data analysis

The data obtained are expressed as *M* ± *SD*. For fatty acid analysis, three data were processed from each muscle sample, so that the sample number in these determinations was 6. The data were statistically analyzed by ANOVA followed by a modified *t*-test according to Bonferroni's method (Wallenstein, Zucker, & Fleiss, 1980). Changes were considered significant when the *p* values were smaller than 0.05.

3 | RESULTS

3.1 | Analysis of muscle fatty acid content

As shown from data seen in Table 1, exposure to linseed-enriched diet resulted in a significant increase in 18:3*n*−3 α −linoleic, 18:2*n*−6 linoleic and 18:1 cis-vaccenic content of beef muscle expressed as fatty acid mg 100 g^{-1} muscle. There were no differences in 18:1 trans, 18:0 stearic, 16:1 cis, 16:0 palmitic, 14:0 myristic and 12:0 lauric acid content in samples from animals in control and linseed-enriched diet-fed animals. Similarly, no significant changes were seen between the two groups in muscle phospholipid content (data not shown).

TABLE 1 Fatty acid content (mg 100 g/muscle) of neutral lipids of m longissimus dorsi from Limousine steers fed on linseed-rich diet

Fatty acids	Control group	Linseed group
Total fatty acids	2,755 ± 296	3,082 ± 311
18:3*n*−3 α-linoleic	11 ± 2	61 ± 5*
18:2*n*−6 linoleic	18 ± 3	26 ± 4*
18:1*n*−9 oleic	1,106 ± 116	1,327 ± 129
18:1 cis-vaccenic	22 ± 4	34 ± 4*
18:1 trans	61 ± 7	75 ± 8
18:0 stearic	520 ± 49	511 ± 56
16:1 cis	112 ± 20	112 ± 18
16:0 palmitic	800 ± 92	811 ± 79
14:0 myristic	100 ± 13	120 ± 14
12:0 lauric	5 ± 0.7	5 ± 1.0

The data are *M* ± *SD* obtained with six animals in each group. *Indicated a significant difference from corresponding "Before ISMN" values. *Significantly different from control at $p < 0.05$.

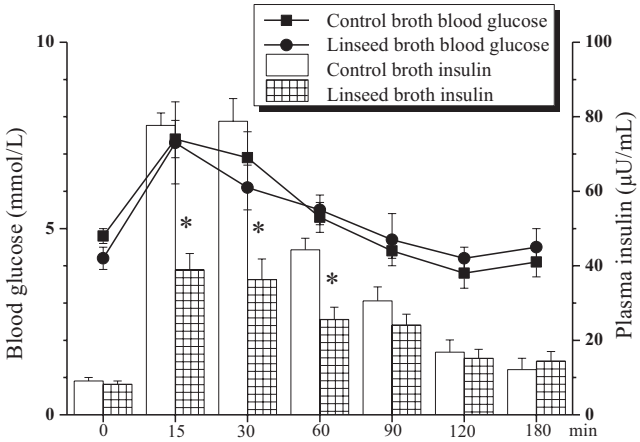

FIGURE 1 Effect of broth made from animals maintained on linseed-rich diet on glucose tolerance test in healthy male volunteers. Black lines show blood glucose values, whereas the columns indicate plasma insulin immunoreactivity. The data are *M* ± *SD* obtained with twelve volunteers. *Indicates a significant difference between "linseed" and control values at $p < 0.05$

3.2 | OGTT and insulin levels

Figure 1 shows the glucose and insulin responses. A 6-day exposure to "linseed soup" attenuated the increase in insulin immunoreactivity in response to oral glucose load as compared to corresponding values after the "control soup" group. There were no differences in either resting or postload plasma glucose levels between the two groups.

4 | DISCUSSION

According to formerly published data, beef fat enriched with PUFA-derivatives may improve insulin sensitivity in rats (Diane et al., 2016). However, extrapolating results from animal studies to humans are not always as easy as it seems (Lalia & Lanza, 2016). Our data presented here show that a daily intake of broth made from meat of animals maintained on lightly bruised linseed-rich forage, is able to inhibit glucose-stimulated insulin release in healthy male volunteers after as short period of broth exposure as 6 days. Since to the best of our knowledge, no reports are available to describe any effect of either liseed-derived eating oil, linseed-based food product, meat, or meat products derived from animals with fatty acids of linseed origin on humans, we consider this result the major finding of the work.

Feeding *n*−3 PUFA supplements as bruised linseed resulted in significant increases in their deposition in muscle lipids (Scollan et al., 2001). The increases were somewhat less than those obtained when formaldehyde-treated, protein-encapsulated lipids were fed (Ashes, Siebert, Gulati, Cuthbertson, & Scott, 1992; Cook, Scott, Faichney, & Davies, 1972). Milk from cows fed formaldehyde-protected linseed oil contained 20% 18:3*n*−3 in the lipid compared with 1% in milk from cows fed unprotected linseed oil. This illustrates well the extent to which ruminant PUFA are amenable to manipulation if they escape rumen biohydrogenation. Our results, however, as seen from data in Table 1, suggest that the seed coat of linseed had some effect in protecting 18:3*n*−3, a component of possible major influence on glucose-stimulated insulin release (Fedor & Kelley, 2009).

Assuming that 100 g/d is an appropriate figure for beef consumption (Enser, Hallett, Hewitt, Fursey, & Wood, 1996), then it is feasible to calculate the contribution of *n*−3 fatty acids to the human diet. Consumption of 100 g/d of beef muscle from cattle fed on linseed, would provide a significant part of the appropriate intake of *n*−3 fatty acids. Hence, beef from the linseed oil treatment could supply most of the daily recommended consumption of *n*−3 fatty. Further enrichment of the *n*−3 fatty acids in beef seems possible, since the deposition of 20:5*n*−3 in Japanese (Koizumi, Suzuki, & Kaneko, 1991) and Australian (Mitchell, Reed, & Rogers, 1991) cattle exceeds the combined 20:5*n*−3 and 22:6*n*−3 deposition in this experiment. Although the levels of 18:1 trans fatty acids were more than doubled as a result of feeding the concentrates containing *n*−3 PUFA, they would not cause the COMA recommendation of a maximum of 2% of calories to be exceeded.

Furthermore, there is doubt that ruminant 18:1 trans, mainly trans-vaccenic acid, is a significant risk factor for cardiovascular disease compared with trans fatty acids arising from chemical hardening of oils. Since the dietary PUFA supplements used in this study also somewhat increased tissue concentrations of conjugated linoleic acid, its reported antiatherogenic action may counteract the effects of increased trans 18:1 fatty acids. Techniques should be developed to protect the *n*−3 PUFA, particularly from linseed, from rumen biohydrogenation and which would incidentally decrease the production of trans fatty acids.

The study was carried out with young, healthy individuals; it is therefore difficult to extrapolate the results obtained to a population with either defined insulin resistance or type II diabetes with or without syndrome ischemic heart disease. The difficulty is further amplified bearing in mind that a glucose load is not a physiological stimulus for insulin release, at least in terms of postprandial hyperinsulinemia. Accordingly, insulin-resistant patients may have altered vascular reactivity with abnormal digestive processes related to the concomitant atherosclerotic disease possibly present even in the pancreas. Moreover, these patients with advanced age are treated with diverse medications, which may be of possible further influence as well. Therefore, the present study only sheds light on a currently less-defined target for functional food candidates of direct or indirect linseed origin being ingested in usual quantities at level of pancreatic beta cells.

ACKNOWLEDGMENTS

This work was supported in part by the 20428-3/2018/FEKUTSTRAT, AGR-PIAC-13-1-2013-0008 and GINOP-2.3.2-15-2016-00062 projects co-financed by the European Union and the European Social Fund.

ORCID

Levente Czegledi http://orcid.org/0000-0002-3947-0208
Bela Juhasz http://orcid.org/0000-0001-8878-5153

REFERENCES

Ashes, J. R., Siebert, B. D., Gulati, S. K., Cuthbertson, A. Z., & Scott, T. W. (1992). Incorporation of n-3 fatty acids of fish oil into tissue and serum lipids of ruminants. *Lipids*, *27*, 629–631. https://doi.org/10.1007/BF02536122

Cavaliere, G., Trinchese, G., Bergamo, P., De Filippo, C., Mattace Raso, G., Gifuni, G., … Mollica, M. P. (2016). Polyunsaturated fatty acids attenuate diet induced obesity and insulin resistance, modulating mitochondrial respiratory uncoupling in rat skeletal muscle. *PLoS ONE*, *11*, e0149033. https://doi.org/10.1371/journal.pone.0149033

Cook, L. J., Scott, T. W., Faichney, G. J., & Davies, H. L. (1972). Fatty acid interrelationships in plasma, liver, muscle and adipose tissues of cattle fed safflower oil protected from ruminal hydrogenation. *Lipids*, *7*, 83–89. https://doi.org/10.1007/BF02532592

Czegledi, L., Tamas, A., Borzsei, R., Bagoly, T., Kiss, P., Horvath, G., … Helyes, Z. (2011). Presence of pituitary adenylate cyclase-activating polypeptide (PACAP) in the plasma and milk of ruminant animals. *General and Comparative Endocrinology*, *172*, 115–119. https://doi.org/10.1016/j.ygcen.2010.12.012

Dewhurst, R., Aston, K., Fisher, W., Evans, R., Dhanoa, M., & McAllan, A. (1999). Comparison of energy and protein sources offered at low levels in grass-silage-based diets for dairy cows. *Animal Science*, *68*, 789–799. https://doi.org/10.1017/S1357729800050815

Diane, A., Borthwick, F., Mapiye, C., Vahmani, P., David, R. C., Vine, D. F., … Proctor, S. D. (2016). Beef Fat enriched with polyunsaturated fatty

acid biohydrogenation products improves insulin sensitivity without altering dyslipidemia in insulin resistant JCR:LA-cp Rats. *Lipids*, *51*, 821–831. https://doi.org/10.1007/s11745-016-4148-7

Dyerberg, J., Bang, H. O., Stoffersen, E., Moncada, S., & Vane, J. R. (1978). Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet*, *2*, 117–119. https://doi.org/10.1016/S0140-6736(78)91505-2

Enser, M., Hallett, K., Hewitt, B., Fursey, G. A., & Wood, J. D. (1996). Fatty acid content and composition of English beef, lamb and pork at retail. *Meat Science*, *42*, 443–456. https://doi.org/10.1016/0309-1740(95)00037-2

Fedor, D., & Kelley, D. S. (2009). Prevention of insulin resistance by n-3 polyunsaturated fatty acids. *Current Opinion in Clinical Nutrition and Metabolic Care*, *12*, 138–146. https://doi.org/10.1097/MCO.0b013e3283218299

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*, *226*, 497–509.

Hay, J. D., & Morrison, W. R. (1973). Positional isomers of Cis and Trans monoenoic fatty acids from ox (steer) perinephric fat. *Lipids*, *8*, 94–95. https://doi.org/10.1007/BF02534337

Jump, D. B., Depner, C. M., & Tripathy, S. (2012). Omega-3 fatty acid supplementation and cardiovascular disease. *Journal of Lipid Research*, *53*, 2525–2545. https://doi.org/10.1194/jlr.R027904

Koizumi, I., Suzuki, Y., & Kaneko, J. J. (1991). Studies on the fatty acid composition of intramuscular lipids of cattle, pigs and birds. *Journal of Nutritional Science and Vitaminology*, *37*, 545–554. https://doi.org/10.3177/jnsv.37.545

Kovacs, P., Szilvassy, Z., Hegyi, P., Nemeth, J., Ferdinandy, P., & Tosaki, A. (2000). Effect of transdermal nitroglycerin on glucose-stimulated insulin release in healthy male volunteers. *European Journal of Clinical Investigation*, *30*, 41–44. https://doi.org/10.1046/j.1365-2362.2000.00588.x

Lalia, A. Z., & Lanza, I. R. (2016). Insulin-sensitizing effects of omega-3 fatty acids: lost in translation? *Nutrients*, *8*, 329.

Mitchell, G. E., Reed, A. W., & Rogers, S. A. (1991). Influence of feeding regimen on the sensory qualities and fatty acid contents of beef steaks. *Journal of Food Science*, *56*, 1102–1103. https://doi.org/10.1111/j.1365-2621.1991.tb14652.x

Rudkowska, I. (2009). Functional foods for health: Focus on diabetes. *Maturitas*, *62*, 263–269. https://doi.org/10.1016/j.maturitas.2009.01.011

Scollan, N. D., Choi, N. J., Kurt, E., Fisher, A. V., Enser, M., & Wood, J. D. (2001). Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. *The British Journal of Nutrition*, *85*, 115–124. https://doi.org/10.1079/BJN2000223

Syngai, G. G., Gopi, R., Bharali, R., Dey, S., Lakshmanan, G. M., & Ahmed, G. (2016). Probiotics - the versatile functional food ingredients. *Journal of Food Science and Technology*, *53*, 921–933. https://doi.org/10.1007/s13197-015-2011-0

Ungvari, E., Monori, I., Megyeri, A., Csiki, Z., Prokisch, J., Sztrik, A., … Benko, I. (2014). Protective effects of meat from lambs on selenium nanoparticle supplemented diet in a mouse model of polycyclic aromatic hydrocarbon-induced immunotoxicity. *Food and Chemical Toxicology*, *64*, 298–306. https://doi.org/10.1016/j.fct.2013.12.004

Wallenstein, S., Zucker, C. L., & Fleiss, J. L. (1980). Some statistical methods useful in circulation research. *Circulation Research*, *47*, 1–9. https://doi.org/10.1161/01.RES.47.1.1

How to cite this article: Bacs Z, Szabo K, Czegledi L, et al. Effect of broth from meat of linseed-fed cattle on glucose-stimulated insulin release in healthy male volunteers. *Anim Sci J*. 2019;90:769–773. https://doi.org/10.1111/asj.13193