Brief Report

Greater impairment of postprandial triacylglycerol than glucose response in metabolic syndrome subjects with fasting hyperglycaemia

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Abstract

Objective: Studies have started to question whether a specific component or combinations of metabolic syndrome (MetS) components may be more important in relation to cardiovascular disease risk. Our aim was to examine the impact of the presence of raised fasting glucose as a MetS component on postprandial lipaemia.

Methods: Men classified with the MetS underwent a sequential test meal investigation, in which blood samples were taken at regular intervals after a test breakfast (t=0 min) and lunch (t=330 min). Lipids, glucose and insulin were measured in the fasting and postprandial samples.

Results: MetS subjects with 3 or 4 components were subdivided into those without (n=34) and with (n=23) fasting hyperglycaemia (≥ 5.6 mmol/l), irrespective of the combination of components. Fasting lipids and insulin were similar in the two groups, with glucose significantly higher in the men with glucose as a MetS component (P<0.001). Following the test meals, there was a higher maximum concentration (maxC), area under the curve (AUC) and incremental AUC (P≤0.016) for the postprandial triacylglycerol (TAG) response in men with fasting hyperglycaemia. Greater glucose AUC (P<0.001) and insulin maxC (P=0.010) was also observed in these individuals after the test meals. Multivariate regression analysis revealed fasting glucose to be an important predictor of the postprandial TAG and glucose response.

Conclusion: Our data analysis has revealed a greater impairment of postprandial TAG than glucose response in MetS subjects with raised fasting glucose. The worsening of postprandial lipaemic control may contribute to the greater CVD risk reported in individuals with MetS component combinations which include hyperglycaemia.

Keywords: postprandial state; non-esterified fatty acids; sequential test meal protocol

Abbreviations: AUC, area under the curve; BMI, body mass index; CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; IAUC, incremental AUC; maxC, maximum concentration; MetS, metabolic syndrome; minC, minimum concentration; LDL-C, low-density lipoprotein cholesterol; NEFA, non-esterified fatty acids; TAG, triacylglycerol; TRL, TAG-rich lipoprotein.

1. Introduction

Coupled with the increasing prevalence of overweight and obesity, 20-25% of adults are now classified with the metabolic syndrome (MetS) which is associated with an increased risk of cardiovascular disease (CVD) and type II diabetes. Studies have started to question whether a specific component or combinations of MetS components are associated with a greater relative risk of CVD than presenting with the syndrome *per se* [1-6]. Three- and four-component combinations highly associated with all cause mortality and cardiovascular events include both abdominal obesity and hyperglycaemia, with the addition of either elevated triacylglycerol (TAG) or blood pressure [2,5]. Pigna and co-workers [7] reported elevated TAG and glucose to be independent predictors of the presence of atherosclerotic plaques. These findings indicate that fasting hyperglycaemia may be an important MetS component in relation to CVD risk.

Dysregulation of TAG in the postprandial state has been associated with insulin resistance, and increasingly recognised as an independent CVD risk factor [8]. Using the DISRUPT database, we have shown a linear trend between the possession of increasing numbers of MetS components and the magnitude of the postprandial TAG and glucose responses [9], with an overall worsening of postprandial lipaemic control in men with 3 and 4/5 components. In the present study, we examined the impact of fasting hyperglycaemia as a MetS component on postprandial TAG, non-esterified fatty acids (NEFA), glucose and insulin responses in men classified with the MetS.

2. Methods

The men included in this DISRUPT dataset (n=57) were from sequential meal postprandial study conducted using the same test meal protocol at the University of Reading between 1997 and 2007. Briefly, these men were non-smokers, free of CVD and diabetes and were not

taking medication known to modify blood lipids or blood pressure [10]. The studies were given a favourable opinion for conduct by the University of Reading Research Ethics Committee and the West Berkshire Health Authority Ethics Committee, and written informed consent was obtained before the studies began.

Subjects were asked to abstain from alcohol and organized exercise regimens on the day prior to the postprandial investigation, and provided with a low-fat evening meal (< 10 g fat). After an overnight fast, subjects consumed a standard test breakfast (4.2 MJ energy, 51 g fat, 125 g carbohydrate and 19 g protein) and lunch (2.6 MJ energy, 30 g fat, 79 g carbohydrate and 15 g protein) at 0 and 330 min respectively, with blood samples taken before and at regular intervals until 480 min after the breakfast. No other food or drink except for water and decaffeinated, sugar-free drinks was allowed during the study day.

Fasted high-density lipoprotein cholesterol (HDL-C) was determined in the supernatant following precipitation with dextran-manganese chloride reagent and low-density lipoprotein cholesterol (LDL-C) was estimated using the Friedewald formula. Plasma lipids and glucose were analysed with an automated analyser (Instrumentation Laboratory (UK) Ltd) using kits supplied by Instrumentation Laboratory and Alpha Laboratories (UK). Insulin was measured by ELISA (Dako Ltd, UK). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using fasting glucose and insulin concentrations [11].

Classification of the MetS was defined retrospectively using the National Cholesterol Education Program Adult Treatment Panel III and International Diabetes Federation definitions [12,13]. As previously described, body mass index (BMI) was used as a substitute for waist circumference [9]. The five MetS components therefore included BMI ≥ 25.7 kg/m^2, fasting glucose ≥ 5.6 mmol/l, TAG ≥ 1.7 mmol/l, HDL-C < 1.03 mmol/l and hypertension (systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 90 mmHg).

Data were analysed using SAS Software, version 9.1.3 (SAS Institute, N.C., USA). Results are presented in the table as mean values ± SD and in the figure as mean values ± SEM. Summary measures of the postprandial response include area under the curve (AUC), incremental AUC (IAUC) and maximum concentration (maxC). For the NEFA response, minimum concentration (minC) was also calculated. An Independent Samples t-test determined differences in baseline characteristics and postprandial summary measures between those with and without raised glucose as a MetS component. Multiple regression analysis was used to determine the independent associations between the MetS components (BMI, blood pressure, fasting TAG, HDL-C and glucose) and the summary measures of the postprandial TAG, glucose and NEFA responses. Partial Eta-squared values were calculated to determine the percentages of variation in summary measures explained by the MetS components. $P \leq 0.05$ was taken as significant.

3. Results

Table 1 summarises baseline characteristics and postprandial summary measures in the group as a whole and according to the presence or absence of glucose as a MetS component. Age, BMI, blood pressure and fasting lipids were not different between the groups with and without fasting hyperglycaemia. By definition, glucose concentrations were significantly higher in men with glucose as a MetS component ($P<0.001$), but insulin and HOMA-IR were not different between the two groups.

Although fasting TAG concentrations were not significantly different, a greater postprandial TAG response was evident in men with fasting hyperglycaemia, which was reflected in the significantly greater maxC (31%), AUC (26%) and IAUC (44%)(Fig. 1a and Table 1). There was a biphasic pattern in the glucose response after the meals, with glucose concentrations falling below baseline levels before ingestion of the second meal (Fig. 1b).

Men with glucose as a MetS component had a significantly greater AUC (8%) for the glucose response, but the IAUC was similar in the two groups (Table 1). Differences were not apparent between groups for the NEFA response.

Insulin was only measured in a subset of men in the postprandial state (n=18/57). A significantly greater insulin maxC (59%) was reached after breakfast in men with fasting hyperglycaemia, with similar insulin responses in the two groups after the second meal (Fig. 1c). AUC and IAUC were not different between the groups (Table 1).

Multivariate regression analysis

Fasting TAG and glucose were positively associated with TAG AUC (TAG *P*=0.002 and glucose *P*=0.024) and maxC (TAG *P*=0.041 and glucose *P*=0.035) whereas BMI was inversely associated with the AUC (*P*=0.0027), IAUC (*P*=0.003) and maxC (*P*=0.0002). Systolic blood pressure was negatively associated with TAG IAUC (*P*=0.044).

Significant independent predictors of the magnitude of the postprandial TAG response (AUC and maxC) were fasting TAG, BMI and glucose, accounting for 15.8%, 14.7% and 8.7% of the variance in AUC and 7.1%, 21.2% and 7.5% of the variance in maxC respectively. BMI was the best predictor of the TAG IAUC accounting for 14.4% of the variance, with systolic blood pressure explaining 7.0% of the variation in this summary measure.

Only fasting glucose was independently associated with the postprandial glucose response, explaining 20% of the variance in both the AUC (*P*=0.0013) and IAUC (*P*=0.0014). For the NEFA response, fasting HDL-C was positively associated with NEFA maxC (*P*=0.0039, Partial Eta-Square 13.7%).

4. Discussion

Our multivariate regression analysis has revealed both confirmatory and novel observations with respect to the impact of individual MetS components on postprandial lipaemia. In agreement with the literature, fasting TAG and BMI were found to be independent predictors of the postprandial TAG response, and fasting glucose the postprandial glucose response in men. Novel findings include the independent association between fasting glucose with TAG AUC and maxC. Specific combinations of MetS components which include hyperglycaemia have been considered to confer a greater CVD risk, but mechanisms underlying these associations are lacking.

Although an exaggerated postprandial TAG response has often been observed in individuals with the MetS [14,15], our study has revealed a reduced ability to handle dietary TAG during the postprandial phase in men with raised fasting glucose as a MetS component compared to those without. Most notably, a marked increase in the postprandial TAG IAUC was observed, with divergence in the incremental responses as early as 180 min after the breakfast meal. Higher TAG concentrations after the second meal, and especially at the end of the postprandial investigation (53% higher at 480 min), suggests a delayed clearance of TAG-rich lipoproteins (TRL) and/or increased production of TRL by the liver (very low density lipoproteins) and intestine (chylomicrons)[16,17]. The lack of an effect of the possession of elevated glucose as a MetS component on the postprandial changes in NEFA, a substrate for TRL-TAG synthesis [18], suggests that a reduced activation of lipoprotein lipase or resistance to the inhibitory actions of insulin on TRL release from the liver and intestine, may have contributed to the greater postprandial TAG response.

Our findings indicate that postprandial glucose handling was not impaired in MetS subjects with raised fasting glucose since incremental glucose responses were similar in the two groups. These observations are in contrast to previous studies which have shown greater post-challenge glucose concentrations after an oral glucose tolerance test in pre-diabetic

individuals with higher fasting glucose levels [19]. The use of sequential composite meals, as opposed to ingestion of a glucose drink alone, represents a more physiological scenario since the fat and protein content of a meal is known to influence the rate of gastric emptying and gut hormone secretion. In our men with hyperglycamia, the higher fasting glucose concentrations may reflect a loss of suppression of hepatic gluconeogenesis, attributable to lower insulin sensitivity. Although fasting insulin was only available in a subset of men (n=41/57), a tendency for a higher HOMA-IR was evident in men with fasting hyperglycaemia but this did not reach significance. However, after the breakfast meal, there was a marked rise in insulin concentrations in these men (59% higher maxC in our subgroup analysis), which we speculate may be an early indicator of a worsening insulin sensitivity. The additional postprandial insulin secretion after the test breakfast may have been sufficient to compensate for the loss of postprandial insulin sensitivity with respect to the glucose and NEFA response, but insufficient to have normalised the TAG response.

In conclusion, our data analysis suggests possession of raised fasting glucose as a MetS component has a greater impact on the postprandial TAG than glucose response. The worsening of postprandial lipaemic control may represent the crucial metabolic defect linking MetS hyperglycaemia with greater CVD risk. Further studies are warranted to confirm these associations and determine the underlying mechanisms of how abnormalities in fasting glucose (and/or insulin) control in the MetS can impact on TAG handling during the postprandial phase.

Author contributions

C.Williams, J.Lovegrove, A.Minihane and K.Jackson designed the postprandial studies, A.Smith and P.Murray downloaded and analysed the postprandial DISRUPT data, and K.Jackson drafted the manuscript with C.Walden. All authors approved the manuscript.

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References

1. Sundstrom J, Vallhagen E, Riserus U, et al. Risk associated with the metabolic syndrome versus the sum of its individual components. Diabetes Care 2006;29:1673-74.
2. Guize L, Thomas F, Pannier B, et al. All-cause mortality associated with specific combinations of the metabolic syndrome according to recent definitions. Diabetes Care 2007; 30:2381-87.
3. Hong Y, Jin X, Mo J, *et al.* Metabolic syndrome, its preeminent clusters, incident coronary heart disease and all-cause mortality – results of prospective analysis for the Atherosclerosis Risk in Communities study. J Intern Med 2007; 262:113-22.
4. Sone H, Tanaka S, Iimuro S, *et al*. Components of metabolic syndrome and their combinations as predictors of cardiovascular disease in Japanese Patients with Type 2 Diabetes. Implications for improved definition. Analysis from Japan Diabetes Complications Study (JDCS). J Atheroscler Thromb 2009; 16:380-387.
5. Franco OH, Massaro JM, Civil J, et al. Trajectories of entering the metabolic syndrome: the framingham heart study. Circ 2009; 120:1943-50.
6. Haring R, Wallaschofski H, Nauck M, et al. Total and cardiovascular disease mortality predicted by metabolic syndrome is inferior relative to its components. Exper Clin Endocrinol Diab 2010; 118:685-91.
7. Pigna G, Napoli A, Zaccagna F, et al. The relationship between metabolic syndrome, its components, and the whole-body atherosclerotic burden as measured by computed tomography angiography. Atherosclerosis 2011; 215:417-20.
8. Jackson KG, Poppit SD, Minihane AM. Postprandial lipaemia and cardiovascular disease risk: interrelationships between dietary, physiological and genetic determinants. Atherosclerosis 2012; 220:22-33.

9. Jackson KG, Walden CM, Murray P, et al. A sequential two meal challenge reveals abnormalities in postprandial TAG but not glucose in men with increasing numbers of metabolic syndrome components. Atherosclerosis 2012; 220:237-43.
10. Jackson KG, Clarke DT, Murray P, et al. Introduction to the DISRUPT postprandial database: subjects, studies and methodologies. Genes Nutr 2009; 5:39-48.
11. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment – insulin resistance and beta-cell function from fasting plasma-glucose and insulin concentrations in man. Diabetologia 1985; 28:412–19.
12. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome - An American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circ 2005; 112:2735-52.
13. Alberti K, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circ 2009; 120:1640-45.
14. Kolovou GD, Anagnostopoulou KK, Pavlidis AN, et al. Metabolic syndrome and gender differences in postprandial lipaemia. Eur J Cardiovasc Prev Rehabil 2006; 13:661-4.
15. Ntyintyane LM, Panz VR, Raal FJ, et al. Postprandial lipaemia, metabolic syndrome and LDL particle size in urbanised South African blacks with and without coronary artery disease. QJM 2008; 101:111-19.
16. Pavlic M, Xiao C, Szeto L, et al. Insulin acutely inhibits intestinal lipoprotein secretion in humans in part by suppressing plasma free fatty acids. Diabetes 2010; 59:580-87.
17. Greenfield M, Kolterman O, Olefsky J, et al. Mechanisms of hypertriglyceridaemia in diabetic patients with fasting hyperglycaemia. Diabetologia 1980; 18:441-446.

18. Xiao C, Lewis GF. Regulation of chylomicron production in humans. Biochim Biophys Acta 2012; 1821:736-46.
19. Ceriello A, Davidson J, Hanefeld M, et al. Postprandial hyperglycaemia and cardiovascular complications of diabetes: An update. Nutr Metab Cardiovas Dis 2006; 16:453-56.

Table 1: Subject characteristics and postprandial summary measures for the lipid, glucose and insulin responses in the whole group of men and according to the presence of fasting glucose as a MetS component

	All	**MetS component combinations**		
	(n=57)	**without glucose** (n=34)	**with glucose** (n=23)	*P*=
Subject characteristics				
Age (y)	54 ± 10	54 ± 11	55 ± 9	0.888
BMI (kg/m^2)	27.6 ± 2.3	27.8 ± 1.8	27.2 ± 2.8	0.271
Blood pressure (mmHg)				
Systolic	141 ± 19	141 ± 15	142 ± 25	0.393
Diastolic	88 ± 10	89 ± 9	87 ± 11	0.495
Fasting biochemical data				
Total cholesterol (mmol/l)	6.49 ± 0.9	6.37 ± 0.9	6.67 ± 0.9	0.224
HDL-C (mmol/l)	1.05 ± 0.2	1.01 ± 0.2	1.10 ± 0.2	0.182
LDL-C (mmol/l)	4.35 ± 0.9	4.29 ± 0.9	4.43 ± 0.9	0.563
TAG (mmol/l)	2.37 ± 0.7	2.28 ± 0.6	2.52 ± 0.8	0.308
NEFA (µmol/l)	486 ± 142	485 ± 142	486 ± 146	0.907

Glucose (mmol/l)	5.48 ± 0.5	5.17 ± 0.3	5.93 ± 0.3	**<0.001**
Insulin (pmol/l)[§]	53.4 ± 28.3	50.9 ± 26.9	56.4 ± 30.2	0.507
HOMA-IR[§]	1.90 ± 1.1	1.70 ± 0.9	2.13 ± 1.2	0.163
Postprandial TAG data				
MaxC (mmol/l)	4.49 ± 1.7	4.00 ± 1.1	5.22 ± 2.0	**0.014**
AUC (mmol/l x 480 min)	1586 ± 537	1434 ± 406	1810 ± 682	**0.016**
IAUC (mmol/l x 480 min)	442 ± 244	375 ± 179	540 ± 294	**0.016**
Postprandial NEFA data				
MinC (µmol/l)	119 ± 12	120 ± 105	117 ± 60	0.757
MaxC (µmol/l)	744 ± 215	738 ± 247	752 ± 160	0.536
AUC_{minC} (mmol/l x min)	158 ± 53	156 ± 62	161 ± 38	0.445
$IAUC_{minC}$ (mmol/l x min)	114 ± 31	111 ± 31	118 ± 32	0.398
Postprandial glucose data				
MaxC (mmol/l)	9.37 ± 1.0	9.18 ± 1.0	9.67 ± 1.1	0.082
AUC (mmol/l x 480 min)	3204 ± 275	3098 ± 250	3360 ± 236	**<0.001**
IAUC (mmol/l x 480 min)	575 ± 262	616 ± 267	516 ± 248	0.159

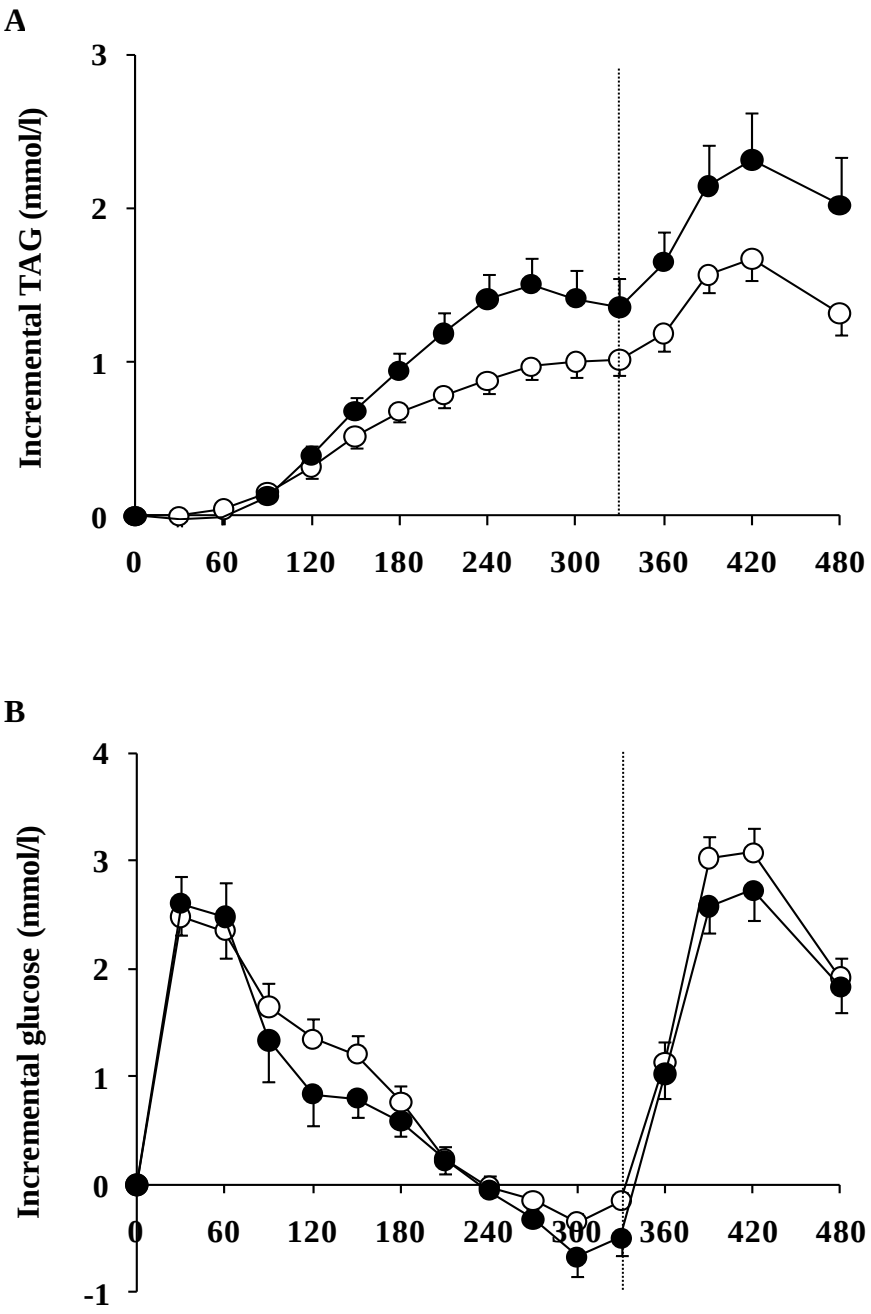
Postprandial insulin response[†]				
MaxC (pmol/l)	736 ± 291	568 ± 172	903 ± 296	**0.010**
AUC (nmol/l x 480 min)	132 ± 45	119 ± 42	145 ± 46	0.215
IAUC (nmol/l x 480 min)	108 ± 42	99 ± 41	117 ± 43	0.371

Values represent mean ± SD. Abbreviations: AUC, area under the curve; BMI, body mass index; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model of assessment-insulin resistance (fasting glucose (mmol/l) x fasting insulin (mU/L)/22.5); IAUC, incremental AUC; maxC, maximum concentration; minC, minimum concentration; LDL-C, low density lipoprotein cholesterol; NEFA, non-esterified fatty acids; TAG, triacylglycerol. For the NEFA response, AUC and IAUC were calculated from minC. [§]The fasting insulin and HOMA data reflects a subset of the group for men in the group as a whole (n=41/57) without glucose (n=22/34) and with raised glucose (n=19/23) as a MetS component. [†]The postprandial insulin data is derived from a subset of n=18/57 men in the group as a whole, n=9/34 men without glucose and n=9/23 with raised glucose as a MetS component.

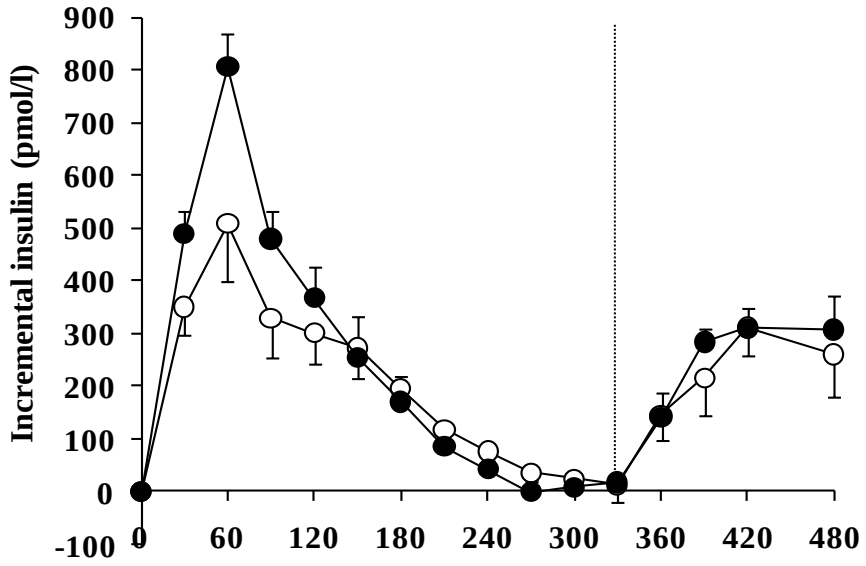
FIGURE LEGEND

Figure 1: Mean ± SEM for the incremental a) triacylglycerol (TAG), b) glucose and c) insulin responses after consumption of a test breakfast (51 g fat, 0 min) and lunch (30 g fat at 330 min) in men without (open circles; TAG and glucose n=34 and insulin n=9) and with (closed circles; TAG and glucose n=23 and insulin n=9) glucose as a MetS component. The dashed line represents the timing of the test lunch (330 min).

FIGURE 1


A
Incremental TAG (mmol/l)
3
2
1
0
0
60
120
180
240
300
360
420
480
B
Incremental glucose (mmol/l)
4
3
2
1
0
-1
0
60
120
180
240
300
360
420
480

C


Incremental insulin (pmol/l)
900
800
700
600
500
400
300
200
100
0
-100
0
60
120
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240
300
360
420
480