Impaired succinate response to a mixed meal in obesity and type 2 diabetes is normalized after metabolic surgery

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ABSTRACT

Objective To explore the meal response of circulating succinate in patients with obesity and type 2 diabetes undergoing bariatric surgery, and to examine the role of gastrointestinal glucose sensing in succinate dynamics in healthy subjects. ***Research Design and Methods*** Cohort I comprised 45 patients with morbid obesity and type 2 diabetes (BMI 39.4±1.9 kg/m^2) undergoing metabolic surgery. Cohort II was a confirmatory cohort of 13 patients (BMI 39.3±1.4 kg/m^2) undergoing gastric bypass surgery. Cohort III comprised 15 healthy subjects (BMI 26.4±0.5 kg/m^2). Cohorts I and II completed a 2-hour meal tolerance test (MTT) before the intervention and at one-year of follow-up, and cohort II also completed a 3-hour lipid test (LT). Cohort III underwent a 3-hour oral glucose tolerance test (OGTT) and an isoglycemic variable glucose infusion (ISO) study.

Results In cohort I, succinate response to MTT at follow-up was greater than before the intervention ($p<0.0001$). This response was confirmed in cohort II with a greater increase after one year of surgery ($p=0.009$). By contrast, LT did not elicit a succinate response. Changes in succinate response were associated with changes in the area under the curve of glucose ($r=0.417$, $p<0.0001$) and insulin ($r=0.204$, $p=0.002$). In cohort III, glycemia *per se* stimulated a plasma succinate response ($p=0.0004$), but its response was greater in the OGTT ($p=0.02$; OGTT *versus* ISO).

Conclusions The meal-related response of circulating succinate in patients with obesity and type 2 diabetes is recovered after metabolic surgery.

Keywords: succinate, obesity, type 2 diabetes, bariatric surgery, incretin effect

A mismatch between nutrient availability and cellular energy requirements is a key contributing factor to the development of obesity and type 2 diabetes mellitus. Dynamic exchanges in intra- and extracellular metabolites are crucial to adequately integrate and coordinate biological networks in cells, particularly the concentration of nutrients and intermediary metabolites (1,2).

There is a wealth of evidence to indicate that succinate is a pleiotropic metabolite functioning not only as an energy intermediary but also as a signaling molecule, both in the cytosol and extracellularly by engaging its cognate receptor SUCNR1 (1,3,4). In the context of energy homeostasis, various signaling roles have been ascribed to succinate, including those of an anti-lipolytic factor (5), a potent activator of brown adipose tissue thermogenesis (6), and a regulator of intestinal gluconeogenesis (7,8). Succinate has also been shown to control the resolution of inflammation, a physiological circuit broken in obesity. Indeed, macrophage-specific deficiency of SUCNR1 in mice stimulates inflammation, glucose intolerance, and cellular metabolic stress (9).

Elevated levels of fasting plasma succinate have been mainly related to pathological processes (10–13), including obesity and type 2 diabetes (12,14). By contrast, a reduction in circulating levels of succinate after bariatric surgery is positively associated with the rate of remission of type 2 diabetes (14). Accordingly, obesity might be associated with succinate resistance, as has been shown for other hormones such as insulin or leptin (15), favoring a vicious cycle of succinate resistance-hypersuccinatemia at least with regards to its effects on the resolution of inflammation (9). Fascinatingly, circulating succinate levels are not only increased in pathology, and it has been known for many years that succinate levels are elevated in some physiological processes such as exercise (16). But, despite much progress, the physiological function of succinate in energy balance and its involvement in the physiopathology of obesity and associated comorbidities is unclear.

The main source of circulating succinate remains enigmatic, although our recent evidence points to the intestine as an important contributor to blood levels (12). To further examine this idea, in the present study, we explored plasma succinate dynamics after food ingestion in patients with morbid obesity and type 2 diabetes before and after bariatric surgery. We also determined whether the succinate response is dependent on glucose and/or lipid sensing, as well as the contribution of glucose sensing through the gastrointestinal tract in healthy subjects.

RESEARCH DESIGN AND METHODS

Cohort I

Cohort I comprised 45 patients with morbid obesity and type 2 diabetes who were submitted to bariatric surgery in the context of a randomized controlled trial (http://www.isrctn.com/ISRCTN14104758). Methodological aspects and the main characteristics of the patients have been published elsewhere (17). In brief, the patients (30 women, 15 men, age 49 ± 8 years, BMI 39.4 ± 1.9 kg/m^2 and HbA1c 7.8 ± 1.9% - 62.0 ± 3.3 mmol/mol) were consecutively recruited for bariatric surgery at the Department of Endocrinology of Bellvitge University Hospital (Barcelona, Spain). Patients were randomly assigned (1:1:1) to three subgroups (n=15) and subjected to one of the following bariatric procedures: laparoscopic greater curvature plication (LGCP), Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG). Before and one year after surgery patients underwent an anthropometric, clinical, and routine biochemical evaluation and a 2-hour mixed-meal tolerance test (MTT).

Cohort II

This group included 13 patients with morbid obesity and type 2 diabetes (9 women, 4 men, age 53 ± 7 years, BMI 39.3 ± 1.4 kg/m^2) undergoing metabolic surgery at Bellvitge University Hospital between June 2016 and June 2017 (**Table 1**). Inclusion/exclusion criteria were the same as those for cohort I. All patients underwent RYGB. The mean weight-loss at the end of follow-up was 33.8% (range 23.8 to 42.3 kg) of the initial weight. Pharmacological treatment was stopped at least 3 days before the functional tests, except insulin treatment, which was stopped 12 hours before tests. As in cohort I, patients from cohort II underwent a complete anthropometric, clinical, and routine biochemical evaluation and a 2-hour MTT before and one year after surgery. Patients also underwent a 3-hour lipid test (LT) during the same periods.

Cohort III

This group included 15 healthy subjects (11 women, 4 men, age 34 ± 12 years, BMI 26.4 ± 1.9 kg/m^2) consecutively recruited at the Hospital Universitari Joan XXIII (Tarragona, Spain). Inclusion criteria were BMI $\geq$ 19.9 $\leq$ 29.9 kg/m^2, absence of acute or chronic systemic disease, absence of pharmacological treatment, and weight stability during the 3 previous months before entry into the study (**Table 2**).

Subjects underwent a 3-hour standard oral glucose tolerance test (OGTT) and, on a separate occasion, a 3-hour isoglycemic glucose infusion (ISO) study using an *ad hoc* algorithm to precisely reproduce the glycemic curve observed during the OGTT (isoglycemic protocol).

Ethical disclosure

All study protocols were conducted according to the principles of the Declaration of Helsinki and approved by the corresponding local ethics committees. All subjects received a comprehensive explanation of the protocol and signed the informed consent before entry into the studies.

Metabolic assessments

Metabolic studies (MTT, LT, OGTT, and ISO) were performed in the morning (starting between 7 and 9 a.m.) after an overnight fast, with no food or drink (except for water) after 8 p.m. of the preceding day. After a medical history record and body composition assessment, an intravenous line was established in the antecubital vein and, after 15 to 30 minutes of rest, the test was started. Specifically, for the ISO study, two intravenous lines were utilized: one in the antecubital vein for the glucose infusion and the other in the cephalic vein (wrist) of the same arm for blood sampling.

Meal tolerance test

Patients ingested a standardized liquid meal beverage (cohort I: Edanec®, Abbott laboratories - 15.9% proteins, 53.8% carbohydrates, and 30.3% lipids [202 kcal] and cohort II: Isosource®, Nestle Health Science - 16% proteins, 49% carbohydrates and 30% lipids [320 kcal]) over 5 minutes. Blood was sampled before meal ingestion (time 0 minutes) and at 15, 30, 60, and 120 minutes after meal ingestion (17).

Lipid test

Patients were prepared as in the MMT protocol. Blood samples were drawn at fasting state (time 0 minutes) and at 60, 120, and 180 minutes after lipid ingestion. The

LT was performed using an oral lipid solution ingested over 5 minutes, containing 50 grams of fat in 100 mL of solution, of which 30% was saturated, 49% was monounsaturated and 21% was polyunsaturated (18).

OGTT and ISO tests

Each volunteer participated in two studies with a 7-to-15-day interval between each. The first study was a 3-hour OGTT (75 g glucose) and plasma glucose was measured every 10 minutes during the test. The OGTT glucose time-curve was then reconstructed in the second, ISO, study using an *ad hoc* algorithm to determine the variable infusion rate of a 20% glucose solution (19). Blood samples for metabolites other than glucose were drawn at -30, 0, 10, 20, 30, 60, 90, 120, 150 and 180 minutes after glucose ingestion or infusion was started.

Determinations

Plasmatic lipid, hepatic and renal profiles were determined by standard enzymatic methods. Plasma glucose was determined by the glucose oxidase method (ADVIA Centaur, Siemens Healthcare, Erlangen, Germany and Analox GM-9, London, UK) Plasma insulin and C-peptide levels were determined by an immunochemiluminometric assay (ADVIA Centaur). Total plasma GLP-1 levels were determined by radioimmunoassay (GLP-1T-36HK) or by ELISA (EZGLP1T-36K) in cohorts I and II/III, respectively (both from Merck KGaa, Darmstadt, Germany) (17). Plasma succinate was determined in plasma filtrates (10.000 kD) using a fluorometric assay (EnzyChromTM Succinate Assay Kit; BioAssay Systems, Hayward, CA) (9,12,14).

Data analysis

Body fat mass of patients (cohort I and II) was estimated using the CUN-BAE equation (20) or was analyzed by bioelectrical impedance (Tanita Europe BV, Amsterdam, The Netherlands) (cohort III). We validated the use of the CUN-BAE index for body fat percentage/adiposity in Cohort II by dual-energy X-ray absorptiometry (DEXA – Hologic QDR 4500; Hologic Inc., Waltham, MA). Fat mass estimated by the CUN-BAE equation correlated with that measured by DEXA ($r=0.913$, $p<0.0001$). Insulin resistance was estimated using the product of fasting plasma glucose (FPG) and triglycerides (TyG index) [$Ln(Triglycerides_{(mg/dl)}*FPG_{(mg/dl)})/2$], or using the oral glucose insulin sensitivity index (OGIS) (21). The insulinogenic index was calculated using the equation ($Insulin_{(\mu U/ml)}$ 30'- 0')/($Glucose_{(mg/dl)}$ 30'- 0'). Area under the time-concentration curve (AUC) was calculated using the trapezoidal rule. Succinate response was calculated as a fold increase of the fasting values. The percentage change of a variable between the baseline and follow-up periods was calculated as follows: $\Delta\%=[(follow\text{-}up\text{-}baseline)/baseline]*100$.

Statistical analysis

All data were tested for normality using the Shapiro-Wilk test. Data are presented as percentage, mean and SD for normally distributed quantitative variables, or median and 25th–75th percentiles (interquartile range [IQR]) for non-normally distributed quantitative variables. Intragroup responses were compared by paired t-test or Wilcoxon signed-rank test when necessary. The time-course (parameter response curves) data were evaluated by ANOVA for repeated measures; *p*-values show the interaction between treatment and time. Correlations between quantitative variables were calculated using

Pearson's or Spearman's test, when necessary. Succinate response was depicted as fold increase from basal values (normalized to 1). Multiple linear regression analysis was used to determine the variables associated with succinate dynamics. All variables significant in univariate analysis were included in the model. Statistical analyses were carried out using SPSS software version 19 (IBM Corp., Armonk, NY).

RESULTS

Dynamic regulation of circulating succinate after food ingestion is dependent on metabolic status

The main anthropometric and clinical characteristics of cohort I have been described previously (14,17), together with the associations between fasting succinate levels and metabolic variables before surgery (14). Notably, we found a positive association between fasting plasma succinate and TyG index, as a measure of insulin resistance (r=0.479, p=0.002), whereas a negative association was observed with the insulinogenic index (r=-0.363, p=0.02). Also, consistent with our previous study (14), fasting plasma succinate levels were reduced by 32.5% at one-year of follow-up (p=0.001) (**Figure 1A**). Follow-up fasting plasma levels of succinate were associated with weight (r=0.386, p=0.01), FPG (r=0.390, p=0.01), HbA1c % (r=0.374, p=0.02), fasting plasma triglycerides (r=0.444, p=0.005) and TyG index (r=0.480, p=0.002).

Of note, an examination of plasma succinate dynamics during an MTT revealed a different pattern before and after surgery. At baseline, nutrient intake resulted in a small but significant increase in plasma succinate of 1.48 ± 0.09-fold over basal levels at 60 minutes (p=0.003). By contrast, a repeat of the MTT one year after surgery revealed a 2.44 ± 0.28-fold increase in succinate over basal levels (p<0.0001) (**Figure 1B**). The

normalization of the AUC fold change of the succinate response by fat mass (kg) confirmed a more pronounced succinate response after surgery, which was independent of the surgical technique (**Figure 1C**). Notably, the percentage change in plasma succinate levels after surgery was associated with the percentage change in plasma glucose ($r=0.417$, $p<0.0001$) and insulin ($r=0.204$, $p=0.002$) during the MTT.

We then performed a multiple regression analysis controlling for age, sex, and change in BMI. Change in AUC of glucose ($\beta=-0.365$, $p=0.02$) and AUC of insulin ($\beta=0.323$, $p=0.03$) appeared as the main determinants of succinate variability. The inclusion of the type of surgical treatment to the model did not change the results.

We sought to confirm the meal-related response of succinate in a second independent cohort (main anthropometric and metabolic variables of cohort II are described in Table 1, and the MTT metabolic response is shown in **Supplemental Figure 1A**). In line with the data of cohort I (14), fasting succinate levels were reduced by 36.0% in cohort II after surgery (**Figure 1D)**. Mirroring the results from cohort I, the MTT (0–60 minutes) showed an increase of succinate 1.22 ± 0.15-fold (p=ns) before surgery and 2.35 ± 0.37-fold ($p=0.004$) in the follow-up analysis (**Figure 1E**). Again, the normalization of the AUC fold change of succinate by fat mass revealed a more pronounced succinate response ($p=0.008$) at follow-up (**Figure 1F**). Of note, in contrast to what was observed in response to an MTT, the hyperlipidemia resulting from the LT did not elicit a succinate response (**Supplemental Figure 1B**).

Meal-related response of succinate is dependent on intestinal glucose sensing

To determine if the nutritional-related succinate response depends on glucose sensing by the gastrointestinal tract or if it is also induced by intravenous glucose infusion, we analyzed succinate dynamics in a cohort of healthy subjects without obesity (cohort III,

anthropometric and metabolic characteristics of subjects are shown in Table 2) after an oral and isoglycemic variable intravenous administration of glucose (OGTT and ISO, respectively).

Plasma glucose curves were superimposable during the tests (p=ns) confirming quite similar peripheral glycemia (**Figure 2A**). As expected, insulinemia was almost 3-fold greater in the oral test than with intravenous glucose stimulation (p<0.0001) (**Figure 2B**). A similar response was observed for C-peptide (p<0.0001, ISO *vs* OGTT) (**Figure 2C**), revealing that gastrointestinal factors account for ~44% of the total insulin response during the OGTT, as expected (19). Also, GLP-1 time-curve analysis demonstrated distinct patterns depending on the route of glucose administration (**Figure 2D**), with oral glucose promptly stimulating GLP-1 release, as previously described (19).

Intriguingly, the succinate response to an oral or intravenous glucose administration differed. Both routes of glucose administration elicited a plasmatic response of succinate with a peak at 60 minutes (**Figure 2E**). In the OGTT, the succinate response was 2.4 ± 0.9-fold higher than the fasting value (p<0.0001), whereas it increased by 1.5 ± 0.4-fold in the ISO (p=0.0004). Accordingly, the AUC for succinate (3 hours) was higher in the OGTT than in the ISO (13020.7 ± 1059.8 *vs* 10140.5 ± 900.4 µmol/L, respectively, p=0.0004).

CONCLUSIONS

To our knowledge, this is the first description of the nutritional modulation of plasma succinate by luminal nutrients, as contrasted with the traditional paradigm of circulating succinate as a pathological metabolic marker (10–12,22,23). Moreover, we demonstrate that the nutritional-related response of succinate is partly dependent on

glucose sensing by the intestine and is associated with the metabolic status of the individual, pointing to an integrated mechanism underpinning these dynamic changes.

Beyond its role as an energy source in the tricarboxylic cycle, succinate is a positive regulator of both intestinal gluconeogenesis (8) and adipose tissue thermogenesis (6). We and others previously demonstrated that increased plasma levels of succinate are associated with metabolic abnormalities such as hypertension, obesity and type 2 diabetes (11,12). Moreover, recovery from hyperglycemia and body weight gain is associated with a reduction in fasting plasma succinate, both by lifestyle changes and bariatric surgery (12,14). Consistent with previous data, we found a clear association between fasting succinate, BMI, HbA1c, FPG and plasma triglycerides, supporting the notion of elevated circulating succinate as a biomarker of a poor metabolic status (9,12,14,24).

When we analyzed the dynamics of circulating succinate in response to a nutritional challenge in patients with morbid obesity and type 2 diabetes, before and after bariatric surgery, we found a similar pattern of results to those of plasma insulin and GLP-1 (17,25,26). In two independent surgical cohorts, we found that the patients had high levels of fasting succinate at baseline (before surgery) and a mostly flat succinate response to a meal test. A study by Sadagopan et al. reported no differences between fasting or postprandial plasma succinate levels in healthy control subjects or patients with diabetes (11). By contrast, a recent metabolomic analysis in healthy postmenopausal women reported a similar succinate response to a mixed meal that observed in our study (24). In our surgical cohorts, weight loss and metabolic improvement promoted by bariatric surgery stimulated a decrease in the fasting levels of succinate and triggered a recovery in nutrient stimulation, with a normal bell-shaped succinate response curve in response to an MTT similar to that observed for glucose, insulin and GLP-1 (17,25,26).

Remarkably, carbohydrates seemed to be uniquely responsible for the succinate response, as the LT had no effect on succinate at baseline or follow-up.

The glucose tests in healthy subjects have shed some light on the potential mechanism underpinning the novel meal-related succinate response. Accordingly, the time-curve of succinate response was clearly higher in the OGTT than in the ISO and is similar to that observed for insulin, C-peptide and GLP-1. The results indicate the relevance of glucose transit through the intestinal tract for post-prandial succinate dynamics, pointing to the intestine as a relevant source of circulating succinate after feeding.

In keeping with this notion, our previous studies demonstrated the close association between circulating succinate and the gut microbiota (12,27). Nevertheless, further studies are required to validate this relationship and alternative sources should not be ruled out, particularly in the context of obesity. For example, it has been described in human adipose tissue explants that hyperglycemia and hypoxia exert a synergistic effect on succinate production (13). Thus, changes in adiposity could explain the differences in succinate response observed between subjects with morbid obesity before and after bariatric surgery. However, the profile of the succinate response was unchanged when the AUC of succinate response was normalized for fat mass (before and after surgery). Similar results were observed with the normalization by lean mass (data not shown). Consequently, it is possible that in obesity circulating succinate originating from both adipose tissue and intestinal microbiota provokes a condition of chronically elevated succinate, suppressing succinate dynamics induced by a nutritional challenge. In fact, weight loss after bariatric interventions improves adipose tissue inflammation (28), gut permeability (29) and dysbiosis, and modifies the levels of TCA intermediary metabolites (30). Hence, it is tempting to speculate that after weight loss, succinate levels decrease

and the evident dynamic response in healthy subjects is recovered, restoring succinate sensitivity, which is a plausible marker of metabolic health status. However, further investigation would be required to determine whether the recovery of succinate response in obese diabetic patients after metabolic surgery is weight loss-dependent, or, conversely, it could be detected in early stages where metabolic improvement does not fully rely on weight loss.

Assessing the physiological significance of nutrient-related succinate dynamics is a key challenge that needs to be addressed in the future. In this context, the new concept of energy metabolites as "signaling molecules" with extracellular functions beyond energy is gaining traction (22,23). Based on the results presented here, it is not unreasonable to suspect that succinate might function similarly to other microbiota-derived metabolites (e.g., short-chain fatty acids) as a paracrine and autocrine signal in metabolic tissues such as adipose tissue (1). Indeed, succinate has been described as an inhibitor of lipolysis in adipocytes *via* activation of SUCNR1 (5,31). It is generally acknowledged that peripheral SUCNR1 remains inactive under healthy conditions and would be activated only by the accumulation of succinate in pathological states. The data presented here and elsewhere describing higher circulating succinate levels after exercise (16) points to a new role for this metabolite in physiological metabolic homeostasis.

In conclusion, our data reveal a meal-related response of circulating succinate that is influenced by the metabolic status of the subject and is dependent on glucose sensing by the gastrointestinal tract. This response is blunted in patients with morbid obesity and type 2 diabetes and is recovered after weight loss. This nutritional modulation of plasma succinate in healthy states goes against the general perception of circulating succinate as an exclusively surrogate marker of hypoxia, tissue damage and inflammation. Further research is needed to establish the physiological role of postprandial succinate and to fully

understand the effect of loss of succinate dynamics in the pathogenesis of diabetes and obesity.

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Table 1 Cohort II: main anthropometric and metabolic variables

Variables	Baseline	Follow-up 12 months	*p**
n (f/m)	13 (9/4)	13 (9/4)	----
Age (years)	53 ± 7	54 ± 7	----
Type 2 diabetes treatment (insulin/others)	13 (4/9)	13 (1/12)	----
BMI (kg/m^2)	39.3 ± 1.4	25.8 ± 2.1	<0.0001
Waist (cm)	124.9 ± 16.4	92.4 ± 11.4	<0.0001
Fasting glucose (mmol/L)	8.9 (6.9 - 11.0)	5.1 (4.5 - 6.1)	0.0007
2 hours glucose (mmol/L)	14.5 ± 5.2	7.0 ± 3.7	0.0005
HbA1c (%)	7.3 (6.7 - 8.0)	5.6 (4.6 - 6.0)	0.0002
HbA1c (mmol/mol)	56.3 (49.7 - 64.5)	37.7 (26.3 - 41.6)	0.0002
Total cholesterol (mmol/L)	4.9 ± 1.0	4.1 ± 0.7	0.002
HDL-cholesterol (mmol/L)	1.2 ± 0.4	1.3 ± 0.3	ns
LDL-cholesterol (mmol/L)	2.9 ± 0.9	2.3 ± 0.6	ns
Triglycerides (mmol/L)	1.7 (1.1 - 3.7)	1.4 (0.7 - 1.5)	0.003
Fasting succinate (µmol/L)	79.7 ± 28.0	51.0 ± 15.3	0.003
Fasting insulin (pmol/L)	116.0 (63.5 - 275.5)	39.0 (34.5 - 55.0)	0.001
Fasting C-peptide (nmol/L)	1.47 ± 0.97	0.52 ± 0.22	0.007
Fasting GLP-1 (pmol/L)	54.6 (42.1 - 72.8)	32.8 (18.5 - 39.6)	0.008
TyG index	5.15 ± 0.12	4.57 ± 0.08	<0.0001
Insulinogenic index	0.3 (0.1 - 0.6)	0.9 (0.3 - 1.3)	ns

Data are presented as mean ± SD or median (25th - 75th percentiles), as appropriate. *p** values for the normal distributed variables were calculated using paired t-test; for the non-normal distributed variables, Wilcoxon signed rank test was used. ns: non-significant

Table 2 Cohort III: main anthropometric and metabolic variables

Variables	OGTT	ISO	*p**
n (f/m)	15 (11/4)	15 (11/4)	----
Age (years)	34 ± 12	----	----
BMI (kg/m^2)	26.4 ± 1.9	----	----
Waist (cm)	86.9 ± 8.3	----	----
Fat mass (%)	30.7 ± 6.3	----	----
Fasting glucose (mmol/L)	5.3 ± 0.3	5.3 ± 0.4	ns
2 hours glucose (mmol/L)	6.8 ± 1.2	6.9 ± 1.0	ns
HbA1c (%)	5.1 ± 0.2	----	----
HbA1c (mmol/mol)	32.0 ± 0.7	----	----
Total cholesterol (mmol/L)	4.1 ± 0.9	----	----
HDL-cholesterol (mmol/L)	1.4 ± 0.2	----	----
LDL-cholesterol (mmol/L)	2.3 ± 0.7	----	----
Triglycerides (mmol/L)	0.7 (0.6 - 1.0)	----	----
Fasting succinate (µmol/L)	41.3 ± 14.4	42.1 ± 4.2	ns
Fasting insulin (pmol/L)	47.5 ± 17.5	43.1 ± 3.8	ns
Fasting C-peptide (nmol/L)	0.4 (0.3 - 0.4)	0.3 (0.2 - 0.4)	ns
Fasting GLP-1 (pmol/L)	26.8 ± 8.7	24.4 ± 2.2	ns
TyG index	4.4 ± 0.1	----	----
OGIS index (mL/min/m^2)	414.0 ± 53.8	----	----
Insulinogenic index	1.0 ± 0.4	----	----

Data are presented as mean ± SD or median (25^{th} - 75^{th} percentiles), as appropriate. *p** values for the normal distributed variables were calculated using paired t-test; for the non-normal distributed variables, Wilcoxon signed rank test was used. ns: non-significant

FIGURE LEGENDS

Figure 1: Succinate response to a meal-tolerance test (MTT). A and D) show fasting values of succinate before and one year after bariatric surgery, respectively, for cohort I and II. B and E) time-curves of plasma succinate response during an MTT (fold increase over basal values), respectively, for cohort I and II. C and F) AUC of the succinate time-curves normalized for fat mass (kg). Data are mean ± SEM. Comparisons were tested using the Wilcoxon signed rank test (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$) and time-curves were compared using repeated measures ANOVA (p-values refer to the interaction between treatment and time).

Figure 2: Cohort III, metabolic response to an oral glucose tolerance test (OGTT) and an isoglycemic variable glucose infusion (ISO) study. A) overlay of plasma glucose curves during OGTT and ISO. B–E) response of plasma insulin, C-peptide, GLP-1 and succinate during the OGTT and ISO. Data are mean ± SEM. Time-curves were compared using repeated measures ANOVA (p-values refer to the interaction between treatment and time).

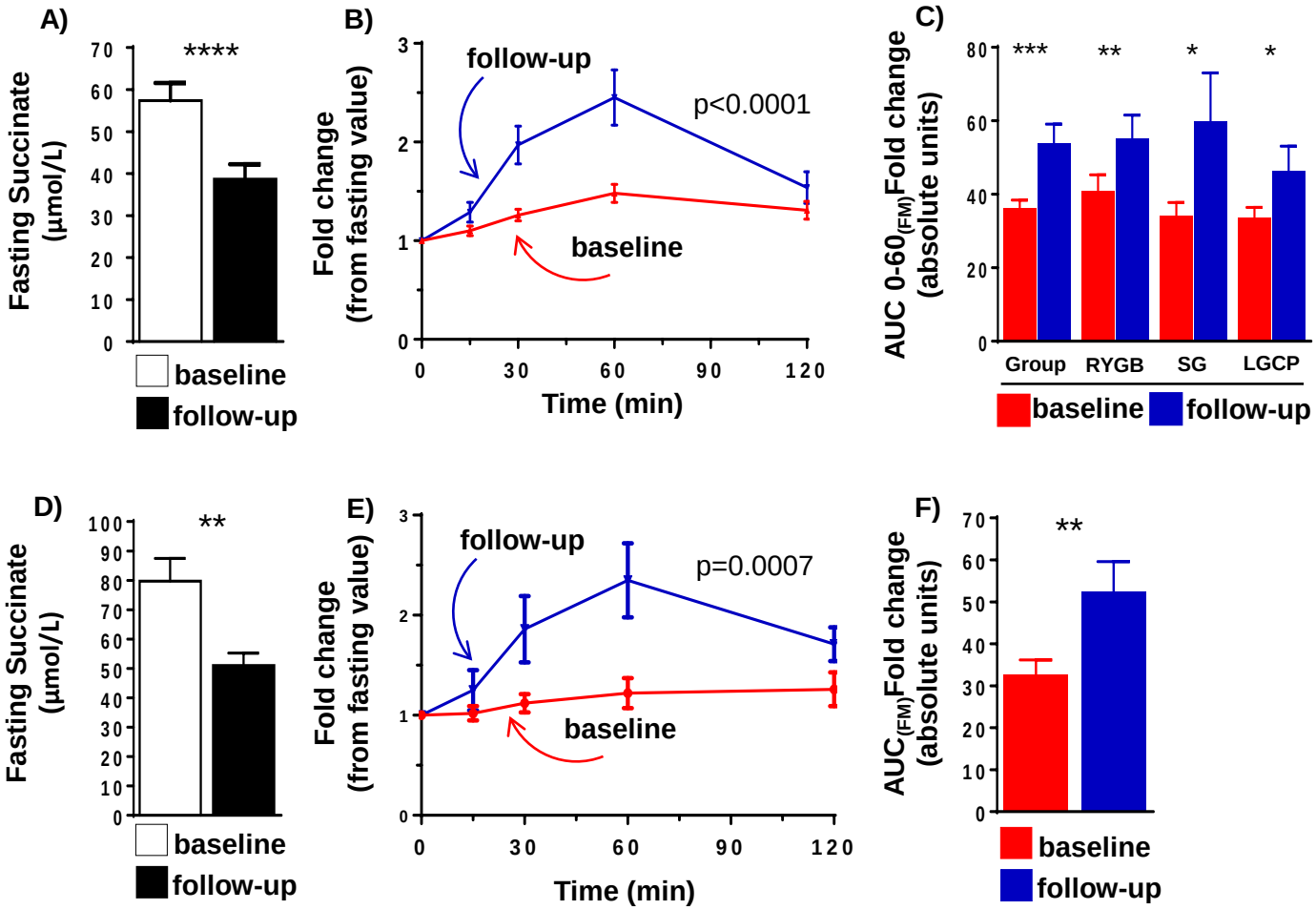

A)
Fasting Succinate (µmol/L)
****
baseline
follow-up
B)
Fold change (from fasting value)
follow-up
p<0.0001
baseline
Time (min)
C)
AUC 0-60(FM)Fold change (absolute units)
***
**
*
*
Group
RYGB
SG
LGCP
baseline
follow-up
D)
Fasting Succinate (µmol/L)
**
baseline
follow-up
E)
Fold change (from fasting value)
follow-up
p=0.0007
baseline
Time (min)
F)
AUC(FM)Fold change (absolute units)
**
baseline
follow-up

Figure 1

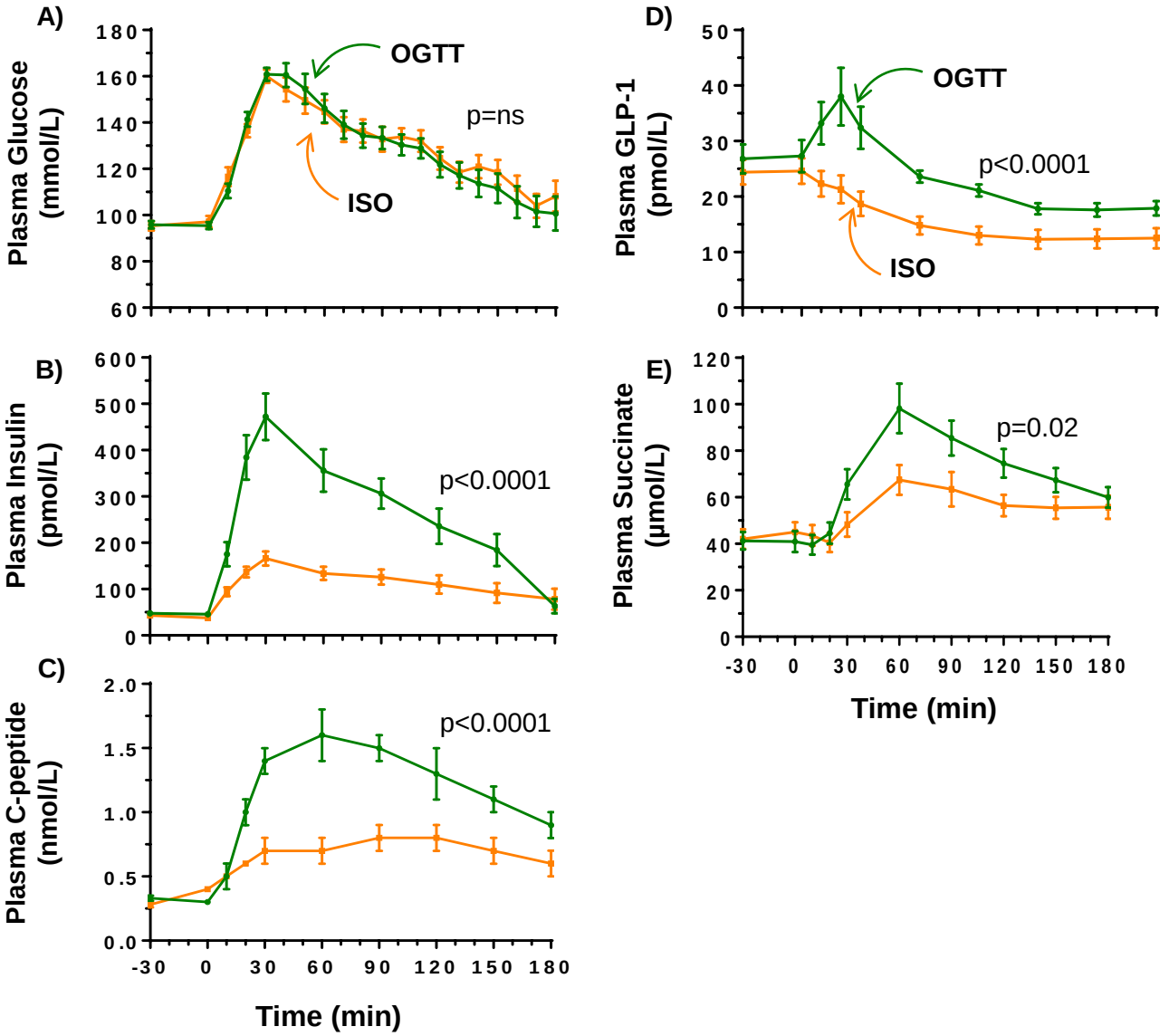
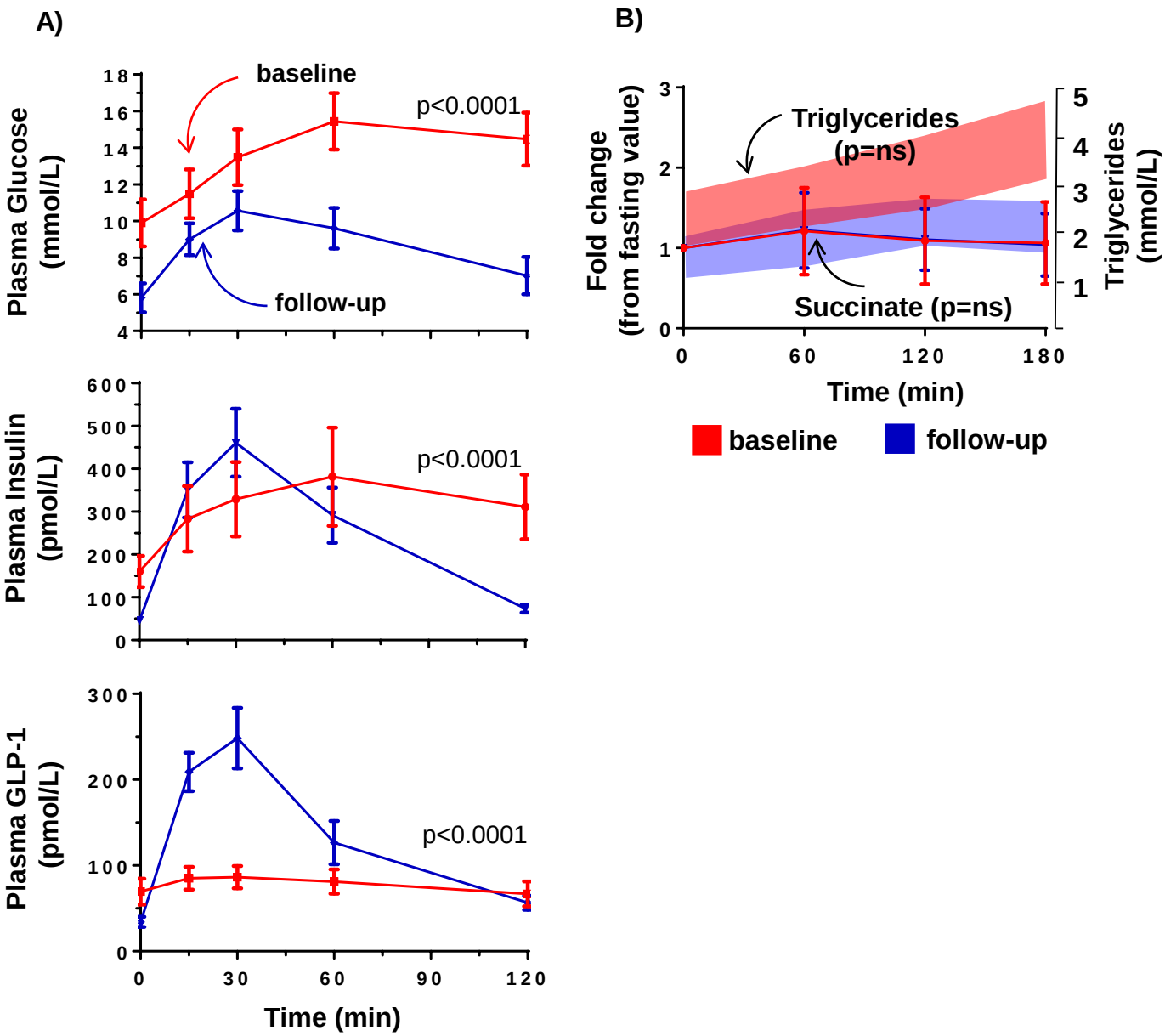

A)
Plasma Glucose (mmol/L)
OGTT
ISO
p=ns
B)
Plasma Insulin (pmol/L)
p<0.0001
C)
Plasma C-peptide (nmol/L)
p<0.0001
Time (min)
D)
Plasma GLP-1 (pmol/L)
OGTT
ISO
p<0.0001
E)
Plasma Succinate (µmol/L)
p=0.02
Time (min)

Figure 2

Supplemental figure 1



Supplemental figure 1: A) metabolic response of cohort II to an MTT. From the top to the bottom, the response of plasma glucose, insulin and GLP-1 to an MTT before and one year after bariatric surgery. B) succinate response of subjects from cohort II to a lipid test (LT). The shadow areas show triglycerides levels during LT, whereas the lines represent the values of plasma succinate. Data are mean ± SD. Time-curves were compared using repeated measures ANOVA (p-values refer to the interaction between treatment and time).