Science Translational Medicine AAAS

www.sciencetranslationalmedicine.org/cgi/content/full/3/80/80re2/DC1

Supplementary Materials for

Differential Metabolic Impact of Gastric Bypass Surgery Versus Dietary Intervention in Obese Diabetic Subjects Despite Identical Weight Loss

Blandine Laferrère,* David Reilly, Sara Arias, Nicholas Swerdlow, Prakash Gorroochurn, Baani Bawa, Mousumi Bose, Julio Teixeira, Robert D. Stevens, Brett R. Wenner, James R. Bain, Michael J. Muehlbauer, Andrea Haqq, Lillian Lien, Svati H. Shah, Laura P. Svetkey, Christopher B. Newgard

*To whom correspondence should be addressed. E-mail: bbl14@columbia.edu

Published 27 April 2011, *Sci. Transl. Med.* **3**, 80re2 (2011)
DOI: 10.1126/scitranslmed.3002043

The PDF file includes:

SUPPLEMENTARY MATERIAL

Materials and Methods

Roux-en-Y Gastric Bypass (GBP) Protocol

For GBP, the jejunum was divided 30 cm from the ligament of Treitz and anastomosed to a 30 ml proximal gastric pouch. The jejunum was reanastomosed 150 cm distal to the gastrojejunostomy. The post-bariatric surgery nutritional recommendations included a daily intake of 600–800 kcal of purée diet, 70 g protein, and 1.8 L fluid for the one month after surgery. This was achieved, on an individual basis, with multiple small meals, snacks, and various commercial protein supplements. The diet after surgery was monitored by food records but not directly supervised. The diet in days preceding the testing was not controlled.

Diet Intervention Protocol

The diet consisted of a meal replacement plan of 1000 kcal/d. A 1-wk supply of meal replacement products (Robard Corp., Mt. Laurel, NJ), including high-protein shakes, bars, fruit drinks, and soups, were given to each patient during an individual weekly visit at the General Clinical Research Center. Fresh fruits and vegetables were allowed. Body weight was measured weekly and the diet adjusted when necessary. If no weight loss, or if weight gain occurred at two consecutive weekly visits, the patients were excluded from the study. Patients were kept on the 1000- kcal diet and in negative energy balance (active weight loss) to achieve a ~10-kg weight loss. Although there was no time limit, the expectation was that patients would lose 10 kg in 4–8 wk. During the weight loss, patients were asked to monitor blood glucose levels by finger stick and keep logs. Diabetes medications were adjusted by a nurse educator or a diabetologist (PI) to avoid hypoglycemia and to fulfill the American Diabetes Association standard of treatment, based on fasting and postprandial glucose levels. In most cases, patients on sulfonylureas had their medication decreased or discontinued to avoid hypoglycemia.

Three-hour oral glucose tolerance test (OGTT)

All patients first underwent a 3-h OGTT with 50 g glucose (noncarbonated, in a total volume of 200 ml). After IV insertion, at 0800 h, subjects received 50 g glucose orally. Blood samples, collected on chilled EDTA tubes with added aprotinin (500 kallikrein inhibitory U/ml blood) and DPP4 inhibitor (LINCO Research, Inc., St. Charles,MO)(10µl/ml blood), were centrifuged at 4^{o}C, and plasma was stored at -70^{o}C.

Assays performed at the Hormone and Metabolite Core Laboratory of the NYONRC.

Total GLP-1, an indicator of GLP secretion, was measured by RIA (LINCO Research) after plasma ethanol extraction. The intra-assay and inter-assay coefficients of variation (CVs) were 3–6.5% and 4.7–8.8%, respectively. This assay cross-reacts 100% with GLP-17–36, GLP-19–36, and GLP-17–37 but does not cross-react with glucagon (0.2%), GLP-2 (<0.01%), or exendin (<0.01%). Total GIP was measured by ELISA. The assay cross-reacts 100% with GIP 1–42 and GIP 3–42 but does not cross-react with GLP-1, GLP-2, oxyntomodulin, or glucagon. The intra-assay and inter-assay CVs were 3.0–8.8% and 1.8–6.1%, respectively. Plasma insulin, C peptide, proinsulin, and glucagon concentrations were measured by RIA (LINCO Research) with an intra-assay CV of 3–8% and inter-assay CV of 5.5–9%. The glucagon assay cross-reacts 100% with glucagon but cross-reacts less than 0.1% with oxyntomodulin. Glucose concentration was measured at the bedside by the glucose oxidase method (Beckman glucose analyzer; Beckman Coulter, Inc., Fullerton, CA).

Statistical Analysis

Total AUCs were calculated using the trapezoidal method. Early phase insulin secretion were calculated as the area under the curve for the first 30 minutes after ingestion of oral glucose (AUC 0–30') or as the difference between insulin levels at 30' and the insulin levels at baseline (Δ 0-30'). Plasma concentrations of C-peptide (AUC0–30' and Δ0-30') were also used to assess the early phase response of the pancreatic beta cells. The homeostasis model assessment of insulin resistance [HOMA-IR; fasting serum insulin (μU/mL) x fasting plasma glucose (mmol / L) / 22.5] was used as an index of insulin resistance, whereas the insulin sensitivity index (ISI) composite was used as an index of whole-body insulin sensitivity and calculated from the OGTT (1) values as follows:

$$ISI = \frac{10,000}{\sqrt{\text{Fasting Glucose} \times \text{Fasting Insulin} \times \text{Mean Glucose} \times \text{Mean Insulin}}}$$

Principal Component Analysis of the NYONRC cohort

Important variables that composed a PC were determined by the variable-component correlation within the component correlation matrix (significance: variable-component correlation >0.400 or <-0.400). The results of the PC analysis and the composition of the two major PCs are described in Fig. S1 and Table S5.

Paired comparison of the PC scores after and before weight loss was performed for each group (GBP and diet intervention), and the changes were compared between groups using independent Student's t-tests. Partial correlations were performed for each main PC against outcome variables, adjusting for group. Separately, regression analyses were run with PC1 and PC2, weight loss, and group (GBP or diet intervention) as predictors of key outcome variables.

Figure S1. Screen plot of principal components analysis in the NYONRC cohort.
Scree plot of principal component analysis, all generated components vs. total Eigen value. Shoulder before PC3 and PC4 marks cut-off for components used for further analysis.

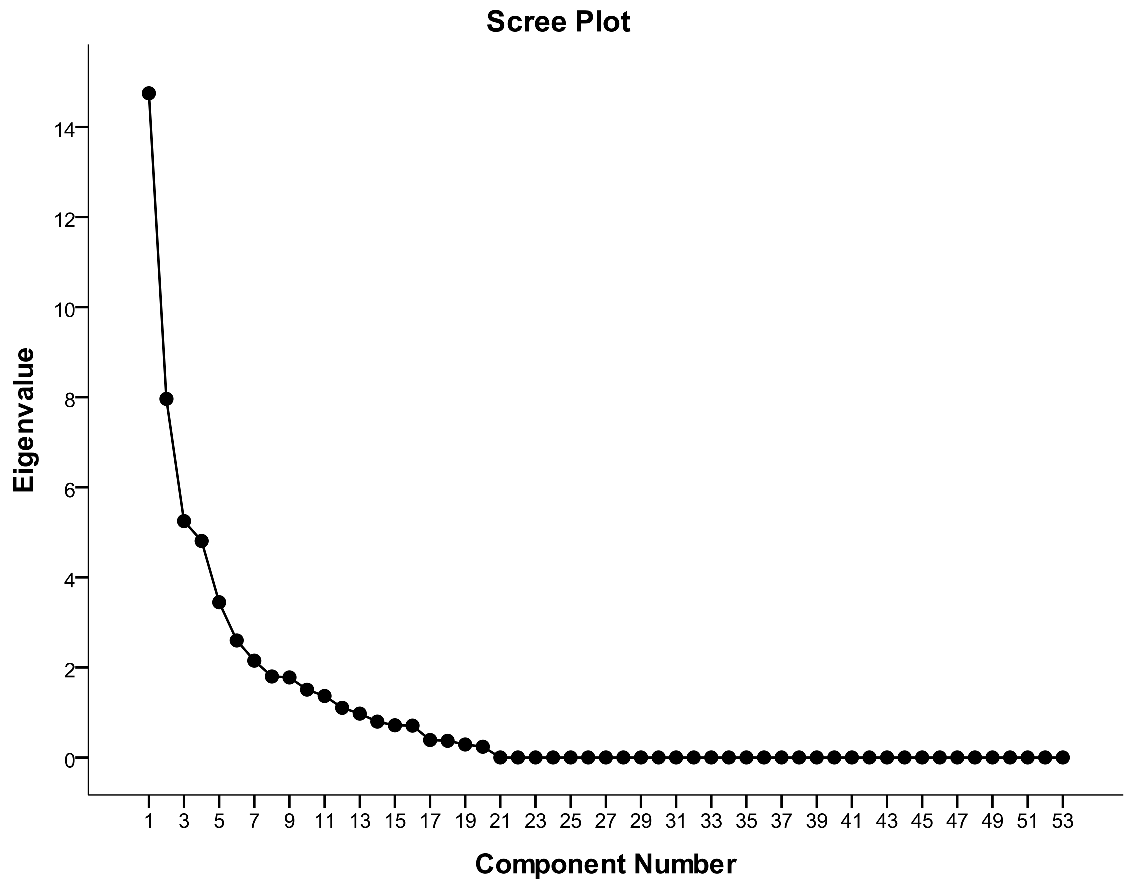

Table S1. Change in plasma acylcarnitine (AC) concentrations after gastric bypass (GBP) and diet intervention in the NYONRC cohort.
The symbol * indicates a significant change in either group (GBP or Diet), *P*<0.05. The reported *P* values are for difference between the changes occurring as a result of GBP versus those occurring as a result of diet.

	Δ Pre-Post-GBP	Δ Pre-Post-Diet	*P*
C2 Acetyl carnitine (μM)	4.13±4.10*	2.53± 2.61*	.295
C3 Propionyl carnitine (μM)	-0.165±0.139*	-0.043± 0.243	.182
C4/Ci4 Butyryl carnitine/ Isobutyryl carnitine (μM)	-0.105±0.118*	-0.024± 0.203	287
C5:1 Tiglyl carnitine (μM)	-0.004±0.039	-0.022± 0.088	.395
C5's Isovaleryl carnitine, 3-methylbutyryl carnitine, 2-methylbutyryl carnitine (μM)	-0.122±0.147*	-0.007± 0.156	.099
C4-OH 3-Hydroxy-butyryl carnitine, β-hydroxy butyryl carnitine (μM)	0.074±0.076*	0.062±0.084*	.746
Ci4-DC/C4-DC **Methylmalonyl carnitine/** **Succinyl carnitine (μM)**	**-0.018±0.020***	**0.022±0.054**	**.039**
C8:1 Octenoyl carnitine (μM)	-0.074±0.135	-0.035± 0.133	.515
C8 Octanoyl carnitine (μM)	0.007±0.099	0.057± 0.15	.387
C5-DC Glutaryl carnitine (μM)	-0.009±0.018	-0.002± 0.022	.418
C6:1-DC/C8:1-OH 3-Hydroxy-cis-5-octenoyl carnitine/ Hexenedioyl carnitine (μM)	-0.007±0.010	-0.000± 0.031	.496
C6-DC Adipoyl carnitine (μM)	0.000±0.039	0.013±0.025	.367
C10:3 **Decatrienoyl carnitine (μM)**	**-0.038±0.027***	**0.014± 0.052**	**.011**
C10:1 Decenoyl carnitine (μM)	0.010±0.088	0.033±0.010	.585
C10 Decanoyl carnitine (μM)	0.013±0.111	0.027±0.140	.806
C8:1-DC Octenedioyl carnitine (μM)	0.004±0.015	0.015±0.032	.328
C10-OH/C8-DC 3-Hydroxy-decanoyl carnitine/ Suberoyl carnitine (μM)	0.018±0.022*	0.013±0.043	.752
C12:1 Dodecenoyl carnitine (μM)	0.007±0.066	0.047±0.089	.265
C12 Lauroyl carnitine (μM)	0.005±0.054	0.040±0.057*	.161
C12-OH/C10-DC 3-Hydroxy-dodecanoyl carnitine/ Sebacoyl carnitine (μM)	0.001±0.005	0.000± 0.007	.816
C14:2	0.003±0.023	0.016± 0.030	.292

Tetradecadienoyl carnitine (μM)			
C14:1 Tetradecenoyl carnitine (μM)	0.021±0.035	0.024±0.038	.814
C14 Myristoyl carnitine (μM)	0.004±0.008	0.006± 0.013	.776
C14:1-OH/C12:1-DC 3-Hydroxy-tetradecenoyl carnitine/ Dodecenedioyl carnitine (μM)	0.001±0.008	0.003± 0.006	.441
C14-OH/C12-DC 3-Hydroxy-tetradecanoyl carnitine/ Dodecanedioyl carnitine (μM)	0.002±0.004	0.001± 0.008	.688
C16:2 Hexadecadienoyl carnitine (μM)	0.002±0.003	0.004± 0.008	.632
C16:1 Palmitoleoyl carnitine (μM)	0.005±0.010	0.009±0.011*	.422
C16 Palmitoyl carnitine (μM)	0.016±0.019*	0.016±0.017*	.989
C16-OH/C14-DC 3-Hydroxy-hexadecanoyl carnitine/ Tetradecanedioyl carnitine (μM)	**0.001±0.003**	**-0.002±0.002***	**.015**
C18:2 Linoleoyl carnitine (μM)	0.013±0.015*	0.017±0.017*	.611
C18:1 Oleyl carnitine (μM)	0.041±0.030*	0.035±0.026*	.665
C18 Stearoyl carnitine (μM)	0.002±0.010	0.000± 0.010	.661
C18:1-OH/C16:1-DC Octadecenedioyl carnitine/ Palmitoleyl carnitine (μM)	0.002±0.003*	0.000± 0.005	.301
C18-OH/C16-DC (μM)	**0.001±0.005**	**-0.001± 0.014**	**.000**
C20:4 Arachidonoyl carnitine (μM)	-0.002±0.002*	-0.001± 0.005	.764
C20 Arachidoyl carnitine, eicosanoyl carnitine (μM)	0.000±0.003	0.001± 0.003	.170
C20:1-OH/C18:1-DC 3-Hydroxy-sphingomyeline/ Octadecanedioyl carnitine (μM)	0.000±0.003	0.003± 0.011	.436
C20-OH/C18-DC 3-Hydroxy-eicosanoyl carnitine/ Octadecanedioyl carnitine (μM)	**0.001±0.004**	**-0.004± 0.007**	**.034**
C22 Behenoyl carnitine/ Docosanoyl carnitine (μM)	**-0.001±0.003**	**0.003± 0.005**	**.039**

Table S2: Characteristics of the Duke cohort at baseline.
Significant differences between groups are shown in bold. Data represent means ± SD. BCAA, branched-chain amino acids—molar sum of leucine + leucine/isoleucine.

	Pre-GBP	Pre-Diet	*P*
Age (yrs)	47.5 ± 7.2	63.5 ± 4.2	0.0008
Weight (kg)	115.0 ± 8.0	127.1 ± 14.3	0.10
Gender (% female)	100%	16.7%	0.29
BMI (kg/m^2)	41.5 ± 2.2	39.8 ± 4.4	0.40
Fasting glucose (mmol/L)	5.05 ± 0.44	5.43 ± 0.66	0.74
Fasting insulin (pmol/L)	172.05±88.75	64.5±27.55	0.02
C3 Propionyl carnitine (µM)	0.478 ± 0.205	0.408 ± 0.089	0.46
C5 Isovaleryl carnitine, 3-methylbutyryl carnitine, 2-methylbutyryl carnitine (µM)	0.102 ± 0.059	0.124 ± 0.051	0.49
BCAA* (µM)	418.38 ± 109.88	457.37 ± 33.91	0.43

Table S3: Changes after similar weight loss in response to GBP or diet intervention in the Duke cohort.
Significant differences between groups are shown in bold. # BCAA, branched-chain amino acids—molar sum of leucine + leucine/isoleucine. * for Student's paired test of change in pre- and post-intervention levels. P-values are given for comparison of changes in GBP vs. diet groups, adjusted for amount of weight lost in the cases of the metabolic variables.

	Δ Pre-Post-GBP	Δ Pre-Post-Diet	*P*
Weight (kg)	-26.6 ± 2.5*	-27.5 ± 2.3*	0.94
BCAA# (µM)	-164.95 ± 109.67*	-54.83 ± 36.93*	0.05
% Change BCAA	-35.8 ± 18.8	-11.9 ± 8.2	0.02
C3 (µM)	-0.111 ± 0.169	-0.083 ± 0.056*	0.73
C5 (µM)	-0.044 ± 0.056	-0.013 ± 0.032	0.28

Table S4. Principal components analysis output for the NYONRC cohort.
Principal components analysis output for PC 1 through 6. Total eigen value, % of variance and cumulative % of variance described for six components selected based on target of ~72% of variance.

Principal Comp.	Eigen value	% of Variance	Cumulative %
1	14.75	27.83	27.83
2	7.96	15.02	42.85
3	5.25	9.90	52.75
4	4.81	9.07	61.83
5	3.45	6.50	68.33
6	2.59	4.90	73.23

Table S5. Principal components (PC) 1 and PC2 composition in the NYONRC cohort. Principal components analysis of the amino acid (AA) and acylcarnitine (AC) variables resulted in 2 major principal components (PC), which explained 42.8% of the total variation in the data set. For each PC, the major contributing variables are listed. Influential variables composing a principal component were determined by the variable-component correlation within the component correlation matrix (Significance: variable-component correlation >.400 or < -.400).

PC1 Eigen Value=14.75	**Factor Score**	**PC2 Eigen value=7.96**	**Factor Score**
Tetradecenoyl Carnitine	0.808	L-Serine	0.823
Dodecenoyl carnitine	0.803	Butyryl carnitine **or** Isobutyryl carnitine	0.798
Lauroyl carnitine	0.803	L-Valine	0.743
Tetradecenoyl carnitine	0.780	Acetyl Carnitine	0.731
3-Hydroxy-decanoyl carnitine **or** Suberoyl carnitine	0.774	L-Leucine **or** L-Isoleucine	0.725
3-Hydroxy-tetradecanoyl carnitine **or** Dodecanedioyl carnitine	0.772	L-Ornithine	0.653
Adipoyl carnitine	0.762	Palmitoyl carnitine	0.639
Octanoyl carnitine	0.757	Propionyl carnitine	0.638
Decanoyl carnitine	0.756	Arachidonoyl carnitine	0.564
3-Hydroxy-dodecanoyl carnitine **or** Sebacoyl carnitine	0.752	Arachidoyl carnitine, eicosanoyl carnitine	0.539
Decenoyl carnitine	0.725	Isovaleryl carnitine, 3-methylbutyryl carnitine **or** 2-Methylbutyryl carnitine	0.518
3-Hydroxy-palmitoleoyl carnitine **or** *cis* -5-Tetradecenedioyl carnitine	0.712	L-Glutamic acid **and** L-glutamate	0.483
Decatrienoyl carnitine	0.711	Stearoyl carnitine	0.477
3-Hydroxy-tetradecenoyl carnitine **or** Dodecenedioyl carnitine	0.710	L-Alanine	0.472

3-Hydroxy-eicosanoyl carnitine **or** Octadecanedioyl carnitine	0.693
Octenedioyl carnitine	0.687
Octadecenedioyl carnitine	0.671
3-Hydroxy-octadecenoyl carnitine	0.663
3-Hydroxy-octadecanoyl carnitine **or** Hexadecanedioyl carnitine, thapsoyl carnitine	0.655
Hexadecadienoyl carnitine	0.602
Oleyl carnitine	0.593
Myristoyl carnitine	0.575
3-Hydroxy-palmitoleoyl carnitine **or** *cis* –5-Tetradecenedioyl carnitine	0.574
Octenoyl carnitine	0.536
Palmitoleoyl carnitine	0.536
Glutaryl carnitine	0.504
3- Hydroxy –*cis*-5-octenoyl carnitine **or** Hexenedioyl carnitine	0.502
Acetyl Carnitine	0.461
Stearoyl carnitine	0.430
Linoleoyl carnitine	0.412
L-Tyrosine	-0.437
L-Phenylalanine	-0.498
L-Methionine	-0.509

Oleyl carnitine	0.448
L-Proline	0.416
L-Tyrosine	0.405

Table S6 Changes of PCs with GBP and diet intervention in the NYONRC cohort.
Changes in the 6 main PCs after GBP and after equivalent weight loss by diet intervention in the NYONRC cohort, with corresponding P value. Reported *P* is for the comparison between GBP and diet groups.

PC	Change with GBP		Change with Diet		*P*
1	74.99 ± 44.56	.000	46.53 ± 42.86	.005	.152
2	**-225.4 ± 142.5**	**.001**	**-42.21 ± 137.63**	**.333**	**.007**
3	-178.63 ± 104.03	.000	-56.12 ± 80.60	.044	**.007**
4	75.15 ± 114.57	.068	-39.66 ± 108.25	.252	.029
5	**-45.09 ± 17.33**	**.000**	**-8.47 ± 20.29**	**.196**	**.000**
6	7.06 ± 59.83	.718	2.59 ± 48.81	.864	.853

References

1. M. Matsuda, R.A. DeFronzo, Insulin sensitivity indices obtained from oral glucose tolerance testing. Comparison with the euglycemic insulin clamp. *Diabetes Care* **22**,1462–1470 (1999)