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ORIGINAL ARTICLE

Molecular mechanisms of diabetes reversibility after bariatric surgery

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Objective: Insulin resistance is a strong biological marker of both obesity and type 2 diabetes. Abnormal fat deposition within skeletal muscle has been identified as a mechanism of obesity-associated insulin resistance. Biliopancreatic diversion (BPD), inducing a massive lipid malabsorption, leads to a reversion of type 2 diabetes. To elucidate the mechanisms of diabetes reversibility, the expression of genes involved in glucose and free fatty acids (FFAs) metabolism was investigated in skeletal muscle biopsies from obese, type 2 diabetic subjects. Peripheral insulin sensitivity and insulin secretion was also measured.
Subjects: Eight Caucasian obese diabetic patients (BMI $52.1 \pm 1.85\,kg/m^2$) were studied before and 3 years after BPD.
Measurements: The mRNA levels were estimated by quantitative real-time reverse transcription polymerase chain reaction (RT-PCR), insulin sensitivity by the euglycemic–hyperinsulinemic clamp and insulin secretion using a model describing the relationship between insulin secretion and glucose concentration.
Results: Whole-body glucose uptake (*M*), normalized by fat-free mass, significantly increased in post-obese subjects ($P<0.0001$). Total insulin output decreased ($P<0.05$) in association with a significant improvement of β-cells glucose sensitivity ($P<0.05$). mRNA levels of FABP3 ($P<0.05$), FACL ($P<0.05$), ACC2 ($P<0.05$), HKII ($P<0.05$) and PDK4 ($P<0.05$) were significantly decreased, while SREBP1c mRNA increased ($P<0.05$) after BPD.
Conclusion: Reversibility of type 2 diabetes after BPD is dependent on the improvement of skeletal muscle insulin sensitivity, mediated by changes in the expression of genes regulating glucose and fatty acid metabolism in response to nutrient availability.
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Keywords: type 2 diabetes; morbid obesity; insulin resistance; biliopancreatic diversion; gene expression

Introduction

Type 2 diabetes mellitus (type 2 DM) has become one of the major global health problem with epidemic proportions.[1] Obesity is moving toward the same direction.[2] Insulin resistance is a prominent biological marker of both obesity and type 2 DM. It defines a condition in which the ability of insulin to stimulate peripheral glucose utilization and to inhibit hepatic glucose production is reduced, so that normoglycemia is maintained by compensatory hyperinsulinemia.[3] The two combined processes, that is, insulin hypersecretion and insulin resistance, contribute to the development of diabetes mellitus, which manifests when the insulin secretory capacity of the β-cell becomes impaired.

Lipids cover a central role in the pathogenesis of insulin resistance. Circulating free fatty acids (FFAs), which are elevated in many insulin-resistant states, have been suggested to contribute to the insulin resistance of diabetes and obesity by inhibiting glucose uptake, glycogen synthesis and glucose oxidation, and by increasing hepatic glucose output.[4] The link between increased circulating FFAs and insulin resistance might involve accumulation of triglycerides (TGs) and fatty acid-derived metabolites (diacylglycerol, fatty acyl-CoA and ceramides) in muscle and liver. Nuclear magnetic resonance spectroscopy has shown a close correlation between intramyocellular TG content (IMTG) and whole-body insulin resistance in patients with obesity and type 2 diabetes.[5]

Fatty acids might affect insulin sensitivity in different ways: the increased membrane fluidity decreases metabolic rate and fatty acid-derived metabolites interfere with insulin

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signalling.[6] Moreover, while IMTG enhances insulin resistance,[7,8] its depletion, as it occurs after a massive lipid malabsorption subsequent to biliopancreatic diversion (BPD), induces a net improvement in insulin sensitivity.[9,10]

Normally, skeletal muscle physiology is characterized by the capacity to utilize both carbohydrate and lipid fuels; the transition between these fuels depends on its energy requirement. Conversely, obese and diabetic patients are characterized by a metabolic inflexibility,[11] which is an impairment in switching between carbohydrates and fat as fuel substrates. In fact, they have a higher lipid oxidation in insulin-stimulated conditions[12] instead of turning their metabolism toward glucose oxidation. Even if the cellular and molecular mechanism of the metabolic inflexibility are not completely understood, functional impairment of mitochondria has recently been proposed as a possible cause.[13] In type 2 diabetic subjects, skeletal muscle mitochondria are smaller and have an impaired bioenergetic capacity.[14]

Recently, BPD has been proposed as one of the potential therapy for diabetes.[15] The effectiveness of BPD in restoring normal glucose tolerance in the majority of diabetic patients seems to depend mainly on enhancement in insulin sensitivity rather then on augmented insulin secretion.[16]

In the attempt to clarify the mechanisms involved in the reversibility of diabetes after BPD, insulin secretion and sensitivity were measured together with the mRNA expression pattern of a cluster of genes encoding the major controlling enzymes and proteins of the fatty acid oxidative pathway in skeletal muscle biopsies from obese and type 2 diabetic patients, either before or after BPD.

Materials and methods

Subjects

The study group consists of eight Caucasian obese diabetic patients (five women and three men, BMI $52.1 \pm 1.85\,kg/m^2$), clinically euthyroid, with no evidence of renal, cardiac or hepatic dysfunction, or other endocrine or nonendocrine disease and non-treated with other drugs than those for diabetes. The onset of diabetes dated 1–3 years and the average glycated hemoglobin was $8.8 \pm 1.5\%$. All patients went through a metabolic investigation after which they underwent BPD, consisting of a partial gastrectomy with a distal Roux-en-Y reconstruction.[17] Three years postoperatively they underwent a second metabolic investigation for the follow-up study.

Biopsies of the vastus lateralis muscle as well as metabolic measurements and blood samples for chemical analysis were performed after an overnight fast during the two investigation days, before and after BPD. All subjects had given written informed consent and the experimental protocol was approved by the Ethical Committee of the Catholic University in Rome.

Body composition

Before and after the surgical procedure, body weight was measured to the nearest 0.1 kg by a beam scale and height to the nearest 0.5 cm using a stadiometer (Holatin, Crosswell, Wales, UK). Total body water was determined using 0.19 Bq of tritiated water in 5 ml of saline solution administered as an intravenous bolus injection.[18] Blood samples were drawn before and 3 h after the injected dose. The disintegrations per minute were counted in duplicate on 0.5 ml of plasma using a β-scintillation counter (model 1600TR; Canberra-Packard, Meriden, CT, USA). Corrections were made (5%) for nonaqueous hydrogen exchange,[19] and water density at body temperature was assumed to be 0.99371 kg/l. Total body water (in kg) was computed as ^3H_2O dilution space (in l) $\times 0.95 \times 0.99371$. The within-person day-by-day coefficient of variation reported for this method was 1.5%.[20]

Euglycemic–hyperinsulinemic clamp procedure

Peripheral insulin sensitivity was evaluated by the 2 h euglycemic–hyperinsulinemic clamp (EHC) procedure.[21] After inserting a cannula in a dorsal hand vein for sampling arterialized venous blood and another one in the antecubital fossa of the contralateral arm for infusions, the subjects rested in a supine position for at least 1 h. They were placed with one hand warmed in a heated air box set at 60°C to obtain arterialized blood samples. Whole-body glucose uptake (*M* value) in micromoles per kilogram of fat-free mass (FFM) per minute was determined during a primed-constant infusion of insulin (at the rate of 6 pmol/kg/min). The fasting plasma glucose concentration was maintained throughout the insulin infusion by means of a variable glucose infusion and blood glucose determinations every 5 min. Whole-body peripheral glucose use was calculated during the last 40-min period of the steady-state insulin infusion.

Insulin secretion

Beta-cell function was assessed by the C-peptide deconvolution method after oral glucose tolerance test (OGTT).[22]

Skeletal muscle biopsies and preparation of total RNA

Muscle biopsies were obtained under local anesthesia from the *vastus lateralis* portion of the quadriceps femoris muscle at 0800 hours in a different section from EHC procedure. Tissue samples were immediately placed in liquid nitrogen and total RNA was extracted from the frozen tissue according to an established procedure.[23] Average yields of total RNA were 0.21 ± 0.08 and $0.18 \pm 0.1\,\mu g/mg$ of muscle (wet weight)in pre- and post-BPD respectively. Total RNA samples were stored at −80°C.

Quantification of mRNAs

The concentrations of the mRNAs corresponding to the genes of interest listed in Table 1 were measured by

quantitative real-time polymerase chain reaction (PCR) using a light cycler (Roche Diagnostics, Meylan, France), as described previously.[24] First-strand cDNAs were first synthesized from 1 μg of total RNA in the presence of 100 U of Superscript II (Invitrogen, Eragny, France) using both random hexamers and oligo (dT) primers (Promega, Charbonnières, France). The real-time PCR was performed in a final volume of 20 μl containing 5 μl of a 60-fold dilution of the room temperature (RT) reaction medium, 15 μl of reaction buffer from the FastStart DNA Master SYBR Green kit (Roche Diagnostics) and 10.5 pmol of the specific forward and reverse primers (Eurobio, Les Ulis, France).

A list of primers and real-time PCR conditions for each mRNA assay is available upon request (vidal@laennec.univ-lyon1.fr). After amplification, a melting curve analysis was performed to verify the specificity of the reaction. For quantification, a standard curve was systematically generated with six different amounts (150–30 000 molecules/tube) of purified target cDNA cloned in the pGEM plasmid (Promega). The analysis was performed using the LightCycler software (Roche Diagnostics). The results were expressed relative to the hypoxanthine phosphoribosyltransferase 1 (*HPRT*) mRNA concentration, a housekeeping gene used as an internal control and measured in each sample by quantitative real-time PCR. A similar concentration of *HPRT* mRNA was found in skeletal muscle before and after BPD (0.22 ± 0.02 vs 0.26 ± 0.04, $P<0.354$).

Blood chemistry

Plasma glucose levels were measured by a glucose-oxidase method (Beckman, Fullerton, CA, USA). Serum immunoreactive insulin was assayed by using microparticle enzyme immunoassay (Abbott, Pasadena, CA, USA). Serum FFAs and TGs were measured by enzymatic, colorimetric methods.

Table 1 Genes involved in the study

ACC2	Acetyl CoA carboxylase-2
AdipoR1	Adiponectin receptor 1
AdipoR2	Adiponectin receptor 2
CPT1	Carnitine palmitoyl CoA transferase 1
CPT2	Carnitine palmitoyl CoA transferase 2
FABP3	Fatty-acid-binding protein 3
FACL	Fatty-acid CoA ligase
FAT/CD36	Fatty-acid translocase
PDK4	Pyruvate dehydrogenase kinase 4
PPARα	Peroxisome proliferator-activated receptor α
PPARβ	Peroxisome proliferator-activated receptor β
UCP 2	Uncoupling protein 2
UCP 3	Uncoupling protein 3
GS	Glycogen synthase
GLUT 4	Glucose transporter 4
HKII	Hexokinase II
SREBP 1c	Sterol regulatory element binding protein 1c
HPRT (as reference gene)	Hypoxanthine phosphoribosyltransferase 1

Statistical analysis

Data are reported as mean$\pm$s.e., unless otherwise specified. Data analyses were performed with SPSS statistical software (SPSS Inc., Chicago, IL, USA). Two-sided $P<0.05$ was regarded as significant.

The Wilcoxon's signed rank test was performed to compare data from the same subjects before and after BPD, adjusting the *P*-values by using Bonferroni method. The distribution of the residuals, testing for normality and checking the linearity assumptions in the model by means of standard scatter plots.

Predictors of insulin sensitivity changes were tested using the Spearman's correlation. Multiple linear regression was, then, used to fit a model to predict insulin sensitivity changes after BPD. Predictor variables considered for this model included FACL mRNA, FABP3 mRNA, *PDK4* mRNA, *HKII* mRNA, *ACC2* mRNA, plasma levels of FFAs and total insulin output. Variables were allowed to enter the models if significant at the <0.05 probability level.

Results

Table 2 summarizes changes in anthropometric variables after BPD operation and Figure 1 represents the mRNA expression of the enzymes and proteins quantified in this study.

The average weight loss observed was in the order of 40%, depending mainly on a massive decrease in fat mass (~52%), whereas FFM reduction accounted for 24%.

Circulating TGs dropped from 2.51 ± 0.30 to 1.43 ± 0.10 mM ($P<0.01$) after BPD as well as plasma FFAs (0.59 ± 0.10 vs 0.23 ± 0.02 mM, $P<0.05$). A similar reduction was observed for total plasma cholesterol (5.66 ± 0.41 vs 3.67 ± 0.43 mM; $P<0.05$). Fasting plasma insulin (120.60 ± 15.54 vs 45.30 ± 6.74 pM, $P<0.01$) and fasting plasma glucose (7.07 ± 0.83 vs 4.05 ± 0.06 mM, $P<0.01$) were both significantly reduced.

EHC showed that whole-body glucose uptake, normalized by FFM, significantly increased in post-obese subjects (25.11 ± 1.72 vs 54.12 ± 2.39 μmol/kg$_{FFM}$/min, $P<0.0001$).

Table 2 Anthropometric characteristics of the subjects

Characteristics	*Before BPD*	*After BPD*	P
Weight (kg)	155.67 ± 8.90	82.38 ± 5.70	<0.001
BMI (kg/m^2)	52.97 ± 1.85	30.80 ± 1.45	<0.001
FFM (kg)	81.50 ± 3.62	62.26 ± 4.70	<0.001
FM (kg)	65.63 ± 2.32	20.11 ± 1.84	<0.001
Triglycerides (mM)	2.51 ± 0.30	1.43 ± 0.10	<0.001
FFAs (mM)	0.59 ± 0.10	0.23 ± 0.02	<0.05
Cholesterol (mM)	5.66 ± 0.41	3.67 ± 0.43	<0.05
Insulin (pM)	126.6 ± 15.54	45.3 ± 6.74	<0.01
Glycemia (mM)	7.07 ± 0.83	4.05 ± 0.06	<0.01
M/FFM (μmol/kg$_{FFM}$/min)	25.11 ± 1.72	54.12 ± 2.39	<0.0001
Insulin output (pmol/min/m^2)	45.28 ± 8.85	21.43 ± 1.66	<0.05

Abbreviations: BMI, body mass index; FFA, free fatty acids; FFM, fat-free mass; FM, fat mass. Values are expressed as mean$\pm$s.e.

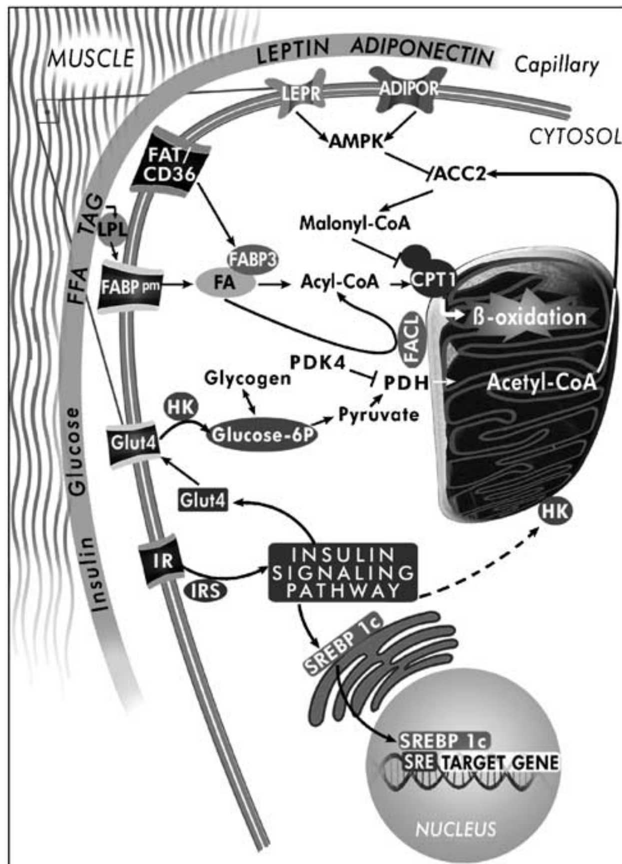

Figure 1 Schematic representation of the main metabolic pathway of energy metabolism in skeletal muscle. FAT/CD36, fatty-acid translocase; FABPpm, plasma membrane fatty acid-binding protein; FABP3, cytosolic fatty acid-binding protein-3; FACL, fatty-acid CoA ligase; CPT1, carnitine palmitoyl CoA transferase; ACC2, acetyl CoA carboxylase-2; PDK4, pyruvate dehydrogenase kinase 4; AdipoR, adiponectin receptor; GLUT4, glucose transporter 4; IR, insulin receptor; SREBP1c, sterol regulatory-binding protein 1c; PDH, pyruvate dehydrogenase; HK, hexokinase; AMPK, AMP-activated protein kinase; LPL, lipoprotein lipase; FA, fatty acid; TAG, triacylglycerol; SRE, SREBP-responsive element; LEPR, leptin receptor.

The insulin output after OGTT significantly decreased in the patients who underwent BPD (45.28 ± 8.85 vs 21.43 ± 1.66 pmol/min/m^2, $P<0.05$). Figure 2 reports the plots of total insulin output against insulin sensitivity. The experimental data were well fitted by a mono-exponential equation ($y = 211.85e^{-0.065x} R^2 = 0.61$ before BPD and $y = 124.2e^{-0.0329x} R^2 = 0.93$ after BPD, $P<0.001$). While the y-intercept halved, the slope of the curve was much stepper, its value being doubled after the operation.

The expression of all the mRNAs in *vastus lateralis* muscle was determined by quantitative real-time RT-PCR before and 3 years after the surgical procedure. The results are reported in Figure 3. The mRNA levels of *FABP3* (87.9 ± 16.7 vs 35.3 ± 5.5, $P<0.05$), *FACL* (10.6 ± 1.7 vs 7.7 ± 1.9, $P<0.05$), *ACC2* (0.74 ± 0.07 vs 0.48 ± 0.07, $P<0.05$), *HKII* (2.92 ± 0.38 vs 1.42 ± 0.18, $P<0.05$) and *PDK4* (131.1 ± 15.9 vs 56.2 ± 10.7, $P<0.05$) were significantly decreased after BPD, while *SREBP1c* mRNA amount increased (2.4 ± 0.2 vs 3.3 ± 0.5, $P<0.05$). The expression of *FAT/CD36*, *CPT1*, *CPT2*, *UCP2*, *UCP3*, *PPARα*, *PPARβ AdipoR1*, *AdipoR2*, *GLUT4* and *GS* mRNAs were not significantly modified after the operation.

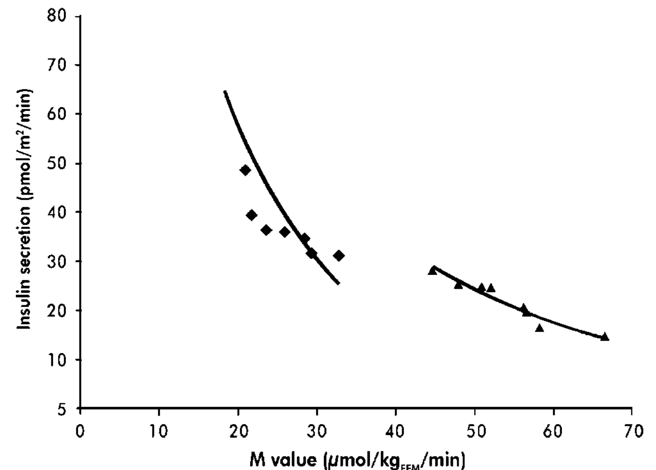

Figure 2 Curvilinear reciprocal relationship between total insulin secretion and insulin resistance (as measured by the euglycemic clamp technique) in the groups of subjects. Solid line and □, before BPD; dashed line and △, 3 years after BPD.

A significant positive correlation (Figure 4) was found between changes of plasma glucose concentrations and changes of *HKII* mRNA levels expressed as delta (pre-post BPD) ($y = 5.32x + 59.15$, $R^2 = 0.2239$, $P<0.05$). Results Delta *PDK4* mRNA correlate directly with delta FFAs ($y = 103.23x – 37.74$, $R^2 = 0.3829$, $P<0.05$, Figure 5a) and inversely with delta glucose uptake values ($y = -0.06x + 22.24$, $R^2 = 0.3109$, $P<0.05$, Figure 5b). Finally, comparison between delta plasma FFAs and delta FACL mRNA levels showed a positive linear correlation ($y = 0.06x – 0.17$, $R^2 = 0.3682$, $P<0.05$, Figure 6).

A multiple regression analysis was used to evaluate the joint effect of changes in *PDK4* mRNA, *HKII* mRNA, *ACC2* mRNA, FACL mRNA, FABP3 mRNA, changes of FFAs and insulin output on the changes of the *M* value. Changes of *PDK4* mRNA amounts ($P = 0.045$) were the one independent variable for predicting *M* variation (Adj $R^2 = 0.93$; $P = 0.043$).

Discussion

This study contributes to elucidate the molecular mechanisms responsible for the reversibility of type 2 diabetes after malabsorptive bariatric surgery. We have already demonstrated in these patients that glucose tolerance normalized at a time when the body mass index was not significantly changed.[15] However, although insulin secretion was fully normalized in glucose normo-tolerant and impaired-glucose tolerant obese individuals, its normalization in type 2

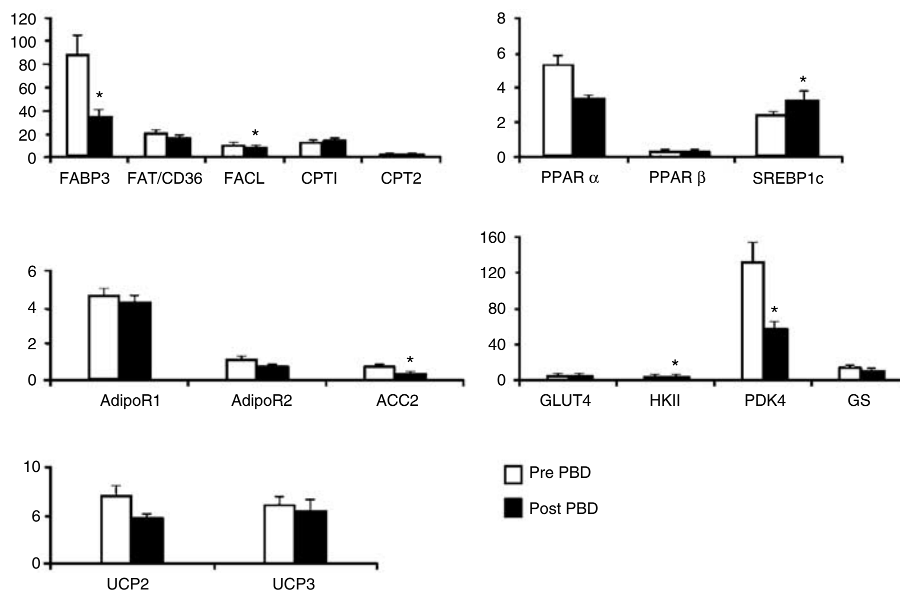

Figure 3 Effect of BPD on the mRNA levels of the target genes in skeletal muscle. Data are means ± s.e. *$P < 0.05$ after vs before BPD.

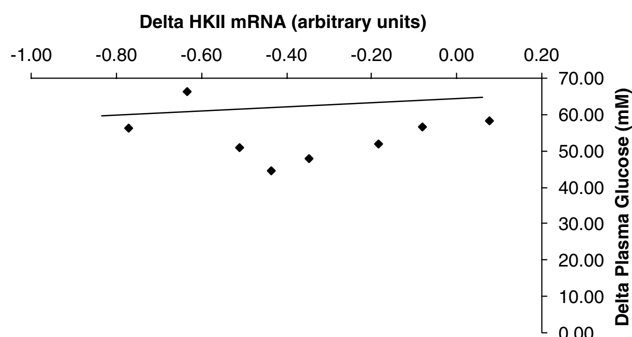

Figure 4 Correlation between delta (before BPD − after BPD values) HKII mRNA and delta plasma glucose.

diabetic subjects was a function of the duration of diabetes.[16] In other words, the β-cell failure in secreting insulin can be only partially reverted if diabetes had a long duration before BPD. On the contrary, tissue insulin sensitivity increased twofold up to normal values[25–28] independently of changes in body weight. In our series, we found that the reversibility of diabetes, as shown by normalization of glycated hemoglobin and plasma glucose levels 2 h after OGTT, depends on the improvement in insulin sensitivity and, as a consequence, on the reduction in insulin secretion. In fact, the sensitivity of β-cell to glucose was increased after the operation as shown in Figure 2 by the shift to the right of the curve fitting the experimental points of insulin secretion as a function of the insulin sensitivity.

Moreover, surgically induced lipid malabsorption, with consequent amelioration of the metabolic profile, is accompanied by changes in key gene expression controlling both lipid and glucose metabolism.

In this study, we have verified a significant decrease in the expression level of *FABP3*, *FACL*, *ACC2*, *PDK4* and *HKII* mRNAs and a significant increase in *SREBP1c* mRNA in skeletal muscle of formerly obese type 2 diabetic patients. The reduction of *PDK4* mRNA is directly correlated to the decrease of plasma FFAs and inversely to glucose uptake values during the clamp. The decrease of *HKII* mRNA levels positively correlate with plasma glucose concentrations as well as with plasma FFAs variations and *FACL* mRNA levels.

Taken altogether, these results reflect metabolic adaptations of the skeletal muscle – which represents the principal site of insulin-mediated glucose disposal[29] – to a specific nutritional state. In fact, after BPD a massive lipid malabsorption intervenes, translating into a reduced availability of fatty acids and into an improved glucose utilization. It is important to consider that while in healthy subjects skeletal muscle is able to switch between carbohydrates and fat as fuel substrates depending on its energy requirement, in insulin-resistant states, such as type 2 diabetes or obesity, this capability is lost. In fact, obese and type 2 diabetic subjects display a higher lipid oxidation in insulin-stimulated conditions[12] instead of turning their metabolism towards glucose oxidation.

The reduced flux of fatty acids observed after BPD might induce an inhibition of *de novo* lipogenesis, together with an increased efficiency in lipid oxidation, as supported by the reduction in *ACC2* mRNA. In fact, if altered *ACC2* expression is responsible for changes in ACC2 protein content and activity, reduced amount of malonyl-CoA, the product of the ACC2 enzyme, removes the inhibition on carnitine palmitoyl transferase I enhancing the long-chain fatty acid-CoA (LCFA-CoA) transportation into the mitochondria, where they are oxidized.[3] Interestingly, increased expression of

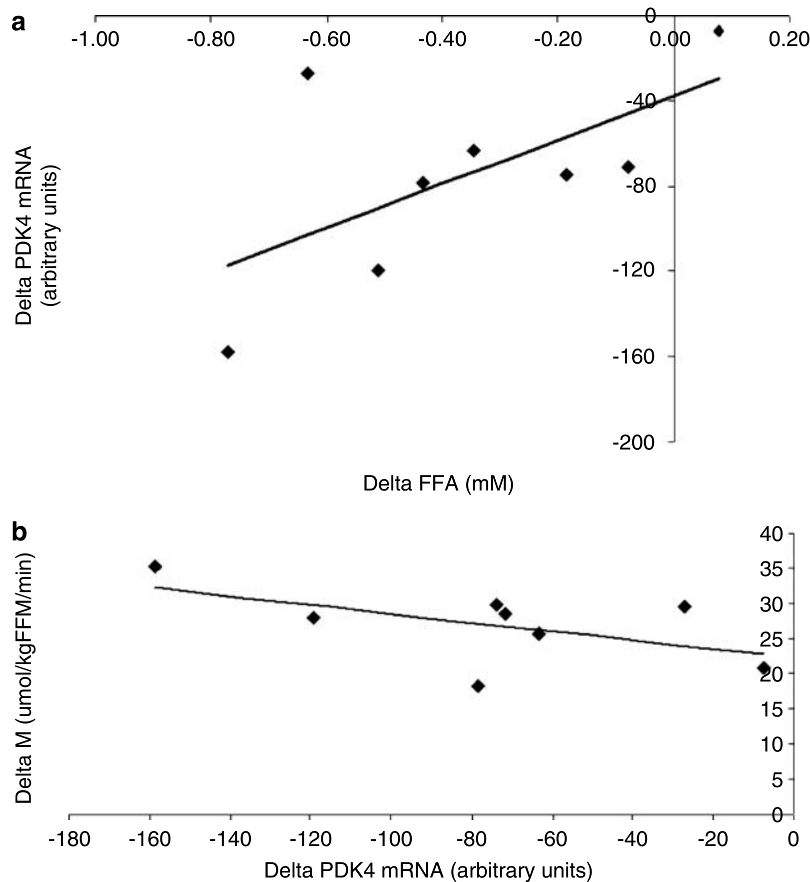

Figure 5 Correlation between delta (before BPD − after BPD values) PDK4 mRNA and delta plasma FFAs (**a**) and delta *M* value (**b**).

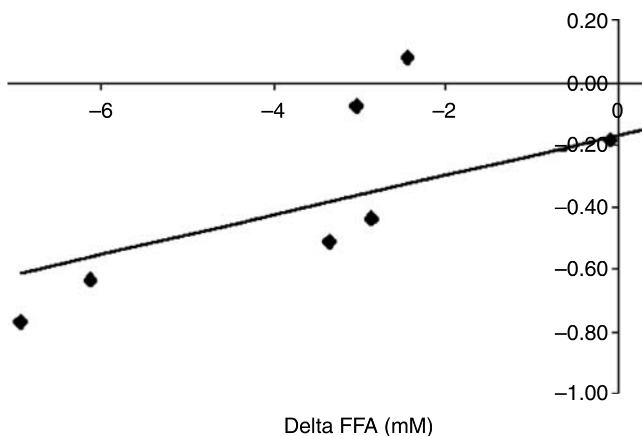

Figure 6 Correlation between delta (before BPD − after BPD values) FACL mRNA and delta plasma FFAs.

ACC2 mRNA has been previously reported in the skeletal muscle of moderately obese subjects.[30] Meanwhile, a decrease of *de novo* fatty acid synthesis, as supported by the reduction in *ACC2* expression, might contribute to a reduction in intramyocellular LCFA- CoA, which have been implicated in the causality of insulin resistance.[31,32] The reduced flux of fatty acids is also in agreement with decreased mRNA expression level of both *FABP3*, the major isoform of cytosolic fatty acid binding protein expressed in skeletal muscle, and *FACL*, the enzyme that catalyzes the activation of LCFA into LCFA-CoA Therefore, the reduced expression of *FACL* mRNA might depend on the lower availability of circulating fatty acids.

Sterol regulatory binding proteins are a family of transcription factors controlling the expression of several enzymes implicated in cholesterol, lipid and glucose metabolism that are transcriptionally regulated by insulin.[33,34] *SREBP1c* is the major isoform presents in skeletal muscle and its expression is reduced in type 2 diabetic subjects.[35] We hypothesize that the improvement of cellular responsiveness to insulin after BPD can restore *SREBP1c* expression.

The reversion of diabetes restores glucose metabolism in formerly obese diabetic patients. In this regard, we have found a decrease in both *HKII* and *PDK4* mRNA levels. Hexokinase II (HKII), by converting glucose to glucose 6-phosphate, helps to maintain the glucose concentration gradient that results in the movement of glucose into the

cells. The reduction in glycaemia after BPD causes a diminished flux of glucose across the cell. It is likely that, in this situation, the balance of cellular glucose availability is ensured by a minimal amount of HKII. In agreement with this hypothesis, we have found a direct relation between plasma glucose concentration and *HKII* gene expression: the lower the amount of the substrate, the lower the quantity of the transcript. Furthermore, the reduced amount of the enzyme does not impair glucose metabolism. Therefore, it is possible that HKII is more active after BPD, perhaps owing to a higher percentage of this isoform docked to the mitochondrial membrane. This hypothesis is supported by data in the literature showing that the activity of HK *in vivo* is much larger in the mitochondrial that in the cytosolic fraction;[36] the transition from one to the other HK form being mediated by insulin.[37] Furthermore, it has been demonstrated that in mice heterozygotes for HKII deficiency, a 50% reduction of both *HKII* mRNA and activity does not impair insulin action or glucose tolerance.[38] Anyway, measurement of the activity of HKII and a deeper study of its regulation in skeletal muscle of diabetic subjects could provide more compelling evidence for this hypothesized mechanism. However, it is important to point out that a decreased expression does not necessarily translate intoa decreased activity. For example, exercise has been demonstrated to increase HKII mRNA expression without changing the activity level of the enzyme.[39]

PDK4 phosphorylates and, hence, inactivates the PDH complex that catalyzes the first irreversible step in glucose oxidation.[40,41] The reduction of *PDK4* mRNA consequent to BPD, if in accordance to reduction of protein level, is consistent with the shift towards carbohydrates oxidation observed after the operation. Before surgery, the increased availability and, consequently, the enhanced oxidation of fatty acids might promote activation of PDK4[42–44] and, thus, inhibit glucose metabolism. After BPD, the strong decrease in fatty acids availability might inhibit the expression of *PDK4*, thus restoring PDH complex function. The correlation of *PDK4* mRNA expression with both plasma FFAs and glucose uptake, strongly supports this hypothesis which is confirmed by the fact that changes of PDK4 levels after BPD are the only factors involved in the improvement of insulin sensitivity.

It might be possible that other mechanisms intervene in the reversibility of diabetes, such as the action of some adipo-cytokines, like adiponectin and leptin. In fact, it is well known that both these hormones stimulate glucose utilization and fatty-acid oxidation in skeletal muscle,[45,46] the latter being regulated through the phosphorylation and activation of 5′-AMP-activated protein kinase (AMPK) which, in turn, inhibits the activity of ACC. We have previously demonstrated that adiponectin circulating levels significantly increase in normotolerant, morbidly obese subjects after BPD.[28] Similarly, the reversion of insulin resistance following BPD allows to the reversal of leptin resistance, the restoration of leptin pulsatility and, subsequently, increased fatty-acid oxidation.[47] However, additional studies are needed to demonstrate a role of such hormones as regulators of genes involved in skeletal muscle energy metabolism. Also fatty acids may control gene transcription as well. Indeed, it has become evident that FFAs can act as signalling molecules involved in regulating gene expression of both lipid and glucose metabolism,[48] mainly acting through the activation of PPARs. Nevertheless, further investigations are necessary to identify the transcription factors involved in the coordinately control of gene expression in response to lipid malabsorption in these subjects.

There is an increasing body of evidence that underlines the importance of increased signals relative to inflammatory cytokines, which stimulate increased deposition of collagen and other lipid metabolites.[49] This would, in turn, interfere with normal insulin signalling. So far, inflammatory signals (inflammatory cytokines and markers such as TNFα, IL1 or CRP) are supposedly responsible for this phenomenon. It is highly likely that the subjects studied in this experiment would be characterized by a decrease in inflammatory markers, and that reduction of adiposity observed in our series may play a role in the reduction of low-grade chronic inflammation. Further sets of studies will be appropriate in order to confirm this hypothesis.

In conclusion, we have shown that reversibility of diabetes in subjects underwent BPD is dependent on the improvement of skeletal muscle insulin sensitivity, mediated by changes in the expression of genes regulating glucose and fatty-acid metabolism in response to nutrient availability.

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