CLINICAL STUDY

Adiponutrin gene is regulated by insulin and glucose in human adipose tissue

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Abstract

Objective: Adiponutrin is a new transmembrane protein specifically expressed in adipose tissue. In obese subjects, short- or long-term calorie restriction diets were associated with a reduction in adiponutrin gene expression. Adiponutrin mRNA level was previously shown to be negatively correlated with fasting glucose plasma levels and associated with insulin sensitivity of non-diabetic obese and non-obese subjects. The purpose of the present work was to get more insight into the regulation of adiponutrin gene expression by insulin and/or glucose using clamp studies and to examine its potential dysregulation in subjects with a deterioration of glucose homeostasis.

Methods: Adiponutrin gene expression was quantified by reverse transcriptase-quantitative PCR in s.c. adipose tissue of healthy lean subjects after an euglycemic hyperinsulinemic clamp (EGHI), a hyperglycemic euinsulinemic clamp, and a hyperglycemic hyperinsulinemic (HGHI) clamp. Adiponutrin gene expression was also analyzed in patients with different levels of insulin resistance.

Results: During EGHI, insulin infusion induced adiponutrin gene expression 8.4-fold ($P=0.008$). Its expression was also induced by glucose infusion, although to a lesser extent (2.2-fold, $P=0.03$). Infusion of both insulin and glucose (HGHI) had an additive effect on the adiponutrin expression (tenfold, $P=0.008$). In a pathological context, adiponutrin gene was highly expressed in the adipose tissue of type-1 diabetic patients with chronic hyperglycemia compared with healthy subjects. Conversely, adiponutrin gene expression was significantly reduced in type-2 diabetics ($P=0.01$), but remained moderately regulated in these patients after the EGHI clamp (2.5-fold increased).

Conclusion: These results suggest a strong relationship between adiponutrin expression, insulin sensitivity, and glucose metabolism in human adipose tissue.

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Introduction

Adipose tissue plays a central role in the regulation of metabolic homeostasis via its dual role as an energy storage and endocrine organ. Indeed, fat cells produce and secrete a number of endocrine and paracrine factors, called adipokines (such as leptin and adiponectin), which profoundly influence metabolism, energy expenditure, and insulin sensitivity (1). Many unsecreted proteins are also expressed in adipose tissue (2), but a lot remains to be identified regarding their role in metabolism.

The recently cloned adiponutrin gene encodes a new unsecreted protein specifically synthesized by the adipocytes. This transmembrane protein is highly induced during adipocyte differentiation (3). Adiponutrin possesses a 'patatin-like' domain, and belongs to a family

of enzymes bearing a transacylase/lipase activity such as for desnutrin/adipose triglyceride lipase (ATGL), GS2 proteins (4–7). Despite the structural homology, the precise biological function of the adiponutrin in adipose tissue remains unclear (8). Several lines of evidence suggest that this protein has no lipase activity (7–9). Furthermore, adiponutrin displays a pattern of expression distinct from the other family members (5, 9). Highly regulated by the nutritional status in rodents, adiponutrin gene expression decreases upon fasting and increases during refeeding in animals fed with a high-carbohydrate diet (3, 10, 11) conversely to the desnutrin/ATGL lipase (5, 9). These observations led to the concept that adiponutrin is more likely involved in the anabolic pathway rather than the lipolytic pathway in adipose tissue (8, 9).

In humans, the regulation of adiponutrin gene expression was unknown until our first study in obese individuals (12). Although adiponutrin mRNA level in adipose tissue was similar in non-obese and in morbidly obese subjects, short- or long-term calorie restriction diets were associated with a marked reduction in adiponutrin mRNA level suggesting an interaction between this adipose-specific protein and metabolic events occurring during modifications of the energy balance. Adiponutrin mRNA level was also negatively correlated with plasma levels of glucose and mildly associated with subject's insulin sensitivity (12). Energy deprivation is classically associated with modulation of insulin sensitivity and glucose metabolism. Interactions between adiponutrin and glucose metabolism were also reported in cellular and animal studies. *In vitro*, the adiponutrin gene is regulated by glucose in 3T3-L1 adipocytes and mildly by insulin (3). *In vivo*, adiponutrin gene expression rapidly increases in the white adipose tissue of rats fed with a high-sucrose diet (10, 11). These studies suggest a regulation between adiponutrin, and glucose and insulin.

Our goal herein was to get more insight into the regulation of adiponutrin expression by glucose and insulin in humans, as well as to investigate its potential abnormal regulation in pathologic conditions associated with altered glucose and insulin homeostasis. To this end, we examined adiponutrin mRNA levels in s.c. adipose tissue during three acute clamp studies with different insulin and/or glucose infusions in healthy males. Adiponutrin gene expression was also investigated in obesity, and type-1 and type-2 diabetes.

Materials and methods

Baseline and stimulated adiponutrin gene expression

Clamp studies in healthy lean subjects Twenty-three lean male volunteers participated in the clamp study (Table 1). Subjects underwent medical examination and routine biological testing. None had a familial or personal history of diabetes and obesity. Subjects were not engaged in physical training (i.e. had <2 h of structured exercise per week) and did not practice exercise for 48 h before the clinical investigation. The study was conducted in the post-absorptive state after an overnight fast. Three groups of lean subjects underwent a 3-h either euglycemic hyperinsulinemic (EGHI), hyperglycemic euinsulinemic (HGEI) or hyperglycemic hyperinsulinemic clamp (HGHI). Subjects who agreed to repeat the clamp study were included in both HGEI and HGHI clamps ($N=5$). The EGHI clamp was performed as described previously (13). Briefly, plasma insulin was elevated to a plateau concentration using primed-constant exogenous

Table 1 Biological parameters of healthy lean subjects before and after the clamps.

	HGEI	EGHI	HGHI
Gender (M)	7	8	8
Age (years)	22±0.6	24±0.9	23±1.1
BMI (kg/m ²)	22.1±0.5	22.6±0.6	23.3±0.5
Triglycerides (mmol/l)	1.2±0.1	1.0±0.1	1.4±0.2
Fasting state			
Insulin (μU/ml)	9.4±0.3*	4.9±0.6	9.0±1.0 [‡]
Glycemia (mmol/l)	5.1±0.1*	4.6±0.1	5.3±0.1 [‡]
Stimulated state			
Insulin (μU/ml)	15.5±1.5 [‡]	152.8±5.8	154.4±9.0
Glycemia (mmol/l)	9.8±0.4 [‡]	4.9±0.3	10.0±0.4 [‡]
Glucose infusion (mg glucose/kg per minute)	4.9±0.8	12.0±0.6	20.3±2.8

HGEI, hyperglycemic euinsulinemic clamp; BMI, body mass index; EGHI, euglycemic hyperinsulinemic clamp; HGHI, hyperglycemic hyperinsulinemic clamp; BMI, body mass index. Two-by-two comparisons show that, before the clamps, differences of insulinemia and glycemia are found in the EGHI group while no difference is observed between HGEI and HGHI groups. * $P<0.05$ comparing EGHI vs HGEI, [‡] $P<0.001$ comparing EGHI vs HGEI, [‡] $P<0.05$ comparing EGHI vs HGHI. Non-parametric Kruskal–Wallis (rank test) test is performed.

insulin infusion at 75 mU/m per minute. Plasma glucose was maintained at fasting levels using 20% dextrose infusion adjusted every 5 min according to the repeated plasma glucose measurements. The HGEI and HGHI clamps were a modification of the technique described by De Fronzo *et al.* (14). The objective of the hyperglycemic clamps was to increase plasma glucose 5.5 mmol/l above the fasting level (measured as the average of three fasting values obtained in 15 min) (15). To achieve that, 20% dextrose was infused in two phases: (i) bolus dose to increase the glycemia to the desired elevated target and (ii) continuous infusion dose to maintain the glycemia at the desired target, which is adjusted every 5–10 min according to the measured plasma glucose concentration. In order to create, simultaneous to hyperglycemia, either euinsulinemic (HGEI) or hyperinsulinemic (HGHI) conditions, endogenous insulin secretion was inhibited using somatostatin analog (Sandostatin, Novartis Pharma, S.A., Switzerland). Sandostatin was infused in two phases: (i) a bolus dose of 25 μg over 1 min given 5 min before the bolus of glucose and (ii) a maintenance dose of 1.0 μg/min (16). Insulin concentration was then replaced either at basal values (HGEI, constant exogenous insulin infusion at a rate of 3.45 mU/m per minute) or a value comparable to that obtained in the EGHI condition (HGHI, primed-constant exogenous insulin infusion at a rate of 75 mU/m per minute) started at the same time of glucose infusion. Potassium phosphate was added to the dextrose in order to maintain normal potassium values under all three clamp conditions.

Baseline adiponutrin gene expression in different pathologic conditions

Subjects Since insulin sensitivity and glucose tolerance vary with age, we explored two independent groups of age-matched subjects. Group 1 (Table 2) comprised ten lean subjects (with no family history of either obesity or type-2 diabetes), nine non-diabetic obese subjects (with no familial history of type-2 diabetes and characterized for glucose tolerance by a classical 75 g 2-h oral glucose tolerance test) and nine type-1 diabetic patients (with no familial antecedent of type-2 diabetes, duration of diabetes being 16 ± 3 years, HbA1c = $9.2\% \pm 0.3$, C peptide < 0.05 ng/ml) with the same age range (22–44) (Table 2). The type-1 diabetic subjects were treated with multiple daily injections of insulin (45 ± 5 IU/day). The last dose of insulin was administered the day before the study. On the morning of the experiment, type-1 diabetic patients showed marked hyperglycemia (12.6 ± 0.8 mM).

Group 2 (Table 3) comprised age-matched older subjects (41–68). We explored seven lean subjects (with no family history of either obesity or type-2 diabetes), seven non-diabetic obese subjects (with no familial history of type-2 diabetes and were characterized for glucose tolerance by a classical 75 g 2-h oral glucose tolerance test) and eight type-2 diabetic patients

(duration of diabetes being 7 ± 1 years, HbA1c = $10.9\% \pm 0.3$). These subjects interrupted their usual treatment of oral anti-diabetic agents at least 1 week before the investigation.

Clamp studies in type-2 diabetic subjects Four type-2 diabetic patients from group 2 agreed to perform a 3-h EGHI.

In all subjects, fasting blood samples were obtained for biochemical and hormonal evaluation. All subjects involved in the different investigation groups gave written consent after being informed of the nature, purpose and possible risks of the study. The experimental protocol was approved by the ethics committee of University of Montreal (Canada) and Hospices Civils de Lyon (France).

Adipose tissue biopsy The adipose tissue pieces were sampled, after an overnight fast, in the s.c. peri-umbilical by needle biopsy under local anesthesia (1% xylocaine). In the clamp study, the s.c. adipose tissue samples were obtained 30 min before the clamp started and at the end (13). Insulin and sandostatin (when applicable) infusions were maintained at the same infusion rate during the post-clamp biopsy. Adipose tissue specimens were immediately frozen in liquid nitrogen and stored at -80°C until analysis.

Table 2 Clinical characteristics of younger lean, obese and type-1 diabetics.

	Lean subjects	Obese subjects	Type-1 diabetics
Gender (F/M)	4/6	5/4	4/5
Age (years)	28 ± 2.0	31 ± 3.0	32 ± 2.5
BMI (kg/m^2)	22.9 ± 0.6	$32.0 \pm 1.1^\dagger$	23.8 ± 0.9
Insulin ($\mu\text{U}/\text{ml}$)	6.3 ± 0.7	$14.7 \pm 2.4^\dagger$	ND
Glycemia (mmol/l)	4.6 ± 0.1	5.0 ± 0.2	$13.4 \pm 1.0^\dagger$
Triglycerides (mmol/l)	0.6 ± 0.06	$1.1 \pm 0.2^\dagger$	$0.4 \pm 0.02^\dagger$
Cholesterol (mmol/l)	4.2 ± 0.3	4.8 ± 0.4	ND
FFA ($\mu\text{mol}/\text{l}$)	383.3 ± 66.4	$506.6 \pm 66.2^*$	$619.4 \pm 99.0^\dagger$
QUICKI	0.373 ± 0.007	$0.327 \pm 0.008^*$	ND

BMI, body mass index; FFA, free fatty acid; QUICKI, quantitative insulin sensitivity check index; ND, not determined. $^*P < 0.05$, $^\dagger P < 0.001$ two-by-two comparison using the Wilcoxon (rank test) are made between lean vs obese subjects or lean vs type-1 diabetics.

Table 3 Clinical characteristics of older lean, obese and type-2 diabetics.

	Lean subjects	Obese subjects	Type-2 diabetics
Gender (F/M)	5/2	3/4	3/5
Age (years)	47 ± 1.3	51 ± 1.9	$56 \pm 2.5^*$
BMI (kg/m^2)	22.8 ± 0.5	$34.7 \pm 1.4^\dagger$	$32.8 \pm 2.0^*$
Insulin ($\mu\text{U}/\text{ml}$)	6.3 ± 0.6	$13.1 \pm 1.8^*$	$13.9 \pm 2.0^*$
Glycemia (mmol/l)	4.9 ± 0.1	5.2 ± 0.2	$11.4 \pm 0.7^\dagger$
Triglycerides (mmol/l)	0.7 ± 0.1	1.2 ± 0.2	$1.6 \pm 0.2^*$
Cholesterol (mmol/l)	5.4 ± 0.4	4.9 ± 0.4	ND
FFA ($\mu\text{mol}/\text{l}$)	647.3 ± 44.1	674.0 ± 36.0	654.5 ± 47.2
QUICKI	0.368 ± 0.008	$0.327 \pm 0.007^*$	$0.293 \pm 0.005^\dagger$

BMI, body mass index; FFA, free fatty acid; QUICKI, quantitative insulin sensitivity check index; ND, not determined. $^*P < 0.05$, $^\dagger P < 0.001$ two-by-comparison using the Wilcoxon (rank test) are made between lean vs obese subjects or lean vs type-2 diabetics.

Analytical procedures Plasma glucose concentrations were measured with a glucose-analyzer (Beckman Glucose analyzer, Mississauga, ON, Canada). Plasma insulin was measured in duplicate with a commercial RIA (Linco Research, St-Charles, MO, USA). In the three clamp conditions, all fasting and post-clamp values were an average of the three time point measurements taken over the period of 30 min. Fasting plasma cholesterol and triglycerides were analyzed on the COBAS INTEGRA 400 (Roche). Serum-free fatty acid values were measured using commercially available kits (WAKO Chemicals, Richmond, VA, USA).

Analysis of mRNA in adipose tissue biopsy Total RNA was extracted from adipose tissue biopsies using the RNeasy total RNA minikit (Qiagen). Adiponutrin mRNA contents were determined by reverse transcriptase-quantitative PCR (RT-qPCR) using a light cycler (Roche), as previously described (17). Results were expressed as the ratio of adiponutrin mRNA to hypoxanthine phosphoribosyltransferase (HPRT) mRNA, a housekeeping gene, used as an internal control. The primer sequences are for the adiponutrin sense 5'-GATGGAGGAGTGAGTGACAA-3' and the adiponutrin antisense 5'-CTGAATGCATCCAAATATCC-3', and for the hypoxanthine phosphoribosyl transferase (HPRT) sense 5'-TTGCTGACCTGCTGGATTAC-3' and HPRT antisenses 5'-AGTTGAGAGATCATCTCCAC-3'. The analysis was performed using the LightCycler software (Roche).

Insulin sensitivity calculation Insulin sensitivity was evaluated using the quantitative insulin sensitivity check index (QUICKI) method, which correlated well with the hyperinsulinemic euglycemic clamp method. Calculations were performed using fasting glucose and insulin as described (18–20).

Statistics analysis The data are expressed as mean $\pm$ S.E.M. Statistical analysis was performed using JMP statistical software (SAS Institute, Inc., Cary, NC, USA). Wilcoxon non-parametric paired test was used to examine the effect of the clamps on adiponutrin mRNA levels and the clinical and metabolic characteristics of the subjects. We used Kruskal–Wallis or Wilcoxon rank test for comparison between independent groups. Non-parametric Spearman's rank correlation was used to examine the correlations of the adiponutrin mRNA levels with the clinical and metabolic characteristics and significance was set at $P < 0.05$.

Results

Adiponutrin gene expression is regulated by insulin and glucose in adipose tissue of healthy lean males

To get insight into the regulation of adiponutrin by insulin and/or glucose in the adipose tissue, healthy lean males were subjected to three types of clamps: HGEI, EGHI, or HGHI. This methodology allowed the investigation of an independent and/or additive effect of glucose and insulin on adiponutrin gene expression. The biological parameters observed before and after the three clamps in these groups of age-matched subjects are shown in Table 1. Some differences in insulinemia in the fasting state were observed in the EGHI group.

Adiponutrin gene expression was significantly enhanced in s.c. adipose tissue of lean subjects in the three clamp conditions (Fig. 1). Compared with each individual basal values, adiponutrin mRNA levels increased during the HGEI clamp 2.2-fold (mean fold change 2.2 ± 0.4 ; $P = 0.03$, Fig. 1A), and 8.4-fold during the EGHI clamp (8.4 ± 1.5 ; $P = 0.008$, Fig. 1B). The HGHI clamp led to a tenfold enhancement of adiponutrin gene expression level (10.0 ± 1.78 compared with basal value; $P = 0.008$, Fig. 1C).

Adiponutrin gene expression is modified in subjects with altered insulin resistance and/or glucose homeostasis

We further investigated adiponutrin gene expression in the adipose tissue of subjects with different levels of insulin sensitivity and glucose tolerance. For appropriate analysis, two independent groups were formed based on significant age differences between lean, obese, and type-1 and type-2 diabetic subjects. Clinical and biological features of young type-1 diabetic patients and age-matched non-diabetic obese and lean subjects (group 1) are presented in Table 2, while those of older non-diabetic obese, type-2 diabetic, and lean subjects (group 2) are shown in Table 3. In both age-matched groups, adiponutrin mRNA expression was similar in lean subjects and obese patients ($P = \text{n.s.}$, Fig. 2A and B), despite the expected decreased insulin sensitivity in obese subjects as evaluated by QUICKI (Tables 2 and 3). This finding is in agreement with our previous data (12). We observed a twofold increase in adiponutrin gene expression in type-1 diabetic subjects when compared either with lean controls or obese subjects ($P = 0.02$, Fig. 2A). In contrast, adiponutrin gene expression was significantly reduced by 50% in the adipose tissue of type-2 diabetic subjects (Fig. 2B). In the group of older lean, obese, and type-2 diabetic subjects, we found a negative correlation between adiponutrin mRNA levels and fasting glycemia ($r = -0.43$, $P = 0.03$). Adjustment for age and sex using multivariate analysis showed similar findings.

Figure 1 Adiponutrin gene expression in response to three acute clamps in s.c. adipose tissue of lean subjects. Adiponutrin mRNA level (μg adiponutrin mRNA/ μg hypoxanthine phosphoribosyl-transferase (HPRT) mRNA) were quantified by real-time reverse transcriptase (RT)-PCR before or after the 3-h clamps in lean males. A paired Wilcoxon signed rank test is performed. The individual induction of Adiponutrin mRNA levels is shown during (A) the hyperglycemic euinsulinemic clamp (HGEI) $^*P=0.03$, (B) the euglycemic hyperinsulinemic clamp (EGHI) $^{\dagger}P=0.008$ and (C) the hyperglycemic hyperinsulinemic clamp (HGHI) $^{\ddagger}P=0.008$. Adiponutrin mRNA level (μg adiponutrin mRNA/ μg HPRT mRNA) were quantified by real time RT-PCR in total preparations.

Adiponutrin gene expression in type-2 diabetics during an hyperinsulinemic clamp study

We evaluated whether the adiponutrin gene expression remained regulated by insulin in the adipose tissue of four type-2 diabetic men (age, 59 ± 3 years; body mass index, 29.9 ± 2.6 kg/m; QUICKI, 0.304 ± 0.01 ; Glucose infusion 3.5 ± 2.3 mg glucose/kg per minute; duration of diabetes = 7 ± 1 years, HbA1c = $10.9\% \pm 0.3$). Despite the decreased expression of adiponutrin found in type-2

Figure 2 Adiponutrin mRNA levels in s.c. adipose tissue of age-matched lean, obese and type-1 diabetic subjects (A) and type-2 diabetic subjects (B). Adiponutrin mRNA levels (μg adiponutrin mRNA/ μg HPRT mRNA) were quantified by real time RT-PCR in total preparations from lean, obese and type-1 diabetic subjects. (A) $^*P=0.007$ when comparing levels of adiponutrin gene expression between type-1 diabetic patients and lean subjects and $P=0.004$ after comparison with obese subjects (Sum rank test). The comparison of Adiponutrin mRNA levels between lean and obese showed no difference. (B) $^*P=0.03$ when comparing levels of adiponutrin gene expression between type-2 diabetics and lean subjects and $P=0.01$ after comparison with obese subjects despite a similar body mass index (BMI) (Sum rank test).

diabetic patients, adiponutrin gene expression was enhanced 2.5-fold after the EGHI clamp (Fig. 3). Although this increase was less than that found in younger lean subjects, the induction in adiponutrin gene expression was significant (mean fold increase, 2.5 ± 0.5 , compared with individual basal values, $P=0.04$) (Fig. 1).

Discussion

The present investigation of different pathophysiological situations suggests a combined role of insulin and glucose in regulating the adiponutrin gene expression in human adipose tissue. Insulin markedly stimulated adiponutrin gene expression and further enhanced the moderate induction by glucose. Chronic hyperglycemia

Figure 3 Adiponutrin gene expression in s.c. adipose tissue of type-2 diabetic patients before and after an acute euglycemic hyperinsulinemic clamp. Adiponutrin mRNA levels (μg adiponutrin mRNA/ μg HPRT mRNA) were quantified by real time RT-PCR in total preparations from type-2 diabetic subjects before or after the 3 h-hyperinsulinemic clamp. * $P=0.04$.

in type-1 diabetics was associated with an increased adiponutrin gene expression, whereas its expression was diminished in the type-2 diabetic state, and not in obesity. Insulin infusion was nevertheless able to stimulate adiponutrin gene expression in diabetic subjects but with less efficiency than in healthy ones.

In a previous investigation, we suggested a potential role for the adiponutrin protein to control energy balance in adipocytes in response to substrate availability. We showed that adiponutrin gene expression positively correlated with the level of insulin sensitivity and negatively with fasting glucose both in obese and non-obese subjects (12). The three clamp conditions performed here allowed some clarifications of the respective *in vivo* effects of insulin and glucose on adiponutrin gene expression. The apparent predominant role of insulin in inducing adiponutrin gene expression in human adipose tissue is in close agreement with the two recent papers showing that (i) in isolated human adipocytes, glucose induction of adiponutrin expression requires insulin and (ii) in mice with insulin or insulin receptor deficiency, lack of insulin stimulation on adipose tissue resulted in impaired adiponutrin expression (9, 21). Interestingly, the levels of insulin reached in our clamp study remained in the range of peak values that would be expected after a large meal and thus could be physiologically relevant (22). *In vitro* experiments in murine 3T3-L1 adipocytes showed that adiponutrin gene expression depended more on glucose than on insulin (3). The moderate glucose induction could be related to a potential effect of sandostatin, which was used to suppress endogenous insulin secretion during the HGEI and HGHI clamp studies. Furthermore, subjects from these two clamp studies also presented higher fasting insulinemia than in subjects from EGHI clamp, which could possibly modulate glucose induced adiponutrin response. Nevertheless, besides species

differences regarding the predominant effector, both *in vivo* and *in vitro* studies suggest that glucose and insulin are important regulators of adiponutrin gene expression in adipocytes. The effect of these effectors on adiponutrin protein expression could not be explored in our study because of the amount of tissues.

Our study also suggests a relationship between the degree of insulin-sensitivity and the modulation of adiponutrin gene expression. A high induction in adiponutrin gene expression was observed in adipose tissue of young type-1 diabetic patients with marked hyperglycemia, but expected unaltered insulin-signaling pathway. Conversely, in type-2 diabetic patients, the deterioration of insulin sensitivity could account for the impaired adiponutrin gene expression in s.c. adipose tissue. Whether genetic variations of adiponutrin also contribute to its gene modulation in diabetics as described in obese subjects is yet to be investigated (21). This could be an attractive hypothesis regarding contribution of an impaired adiponutrin production in insulin resistance process. Clamp studies provide tracks for the role of several adipose tissue-produced proteins in this context. Clearly, the biological function of adiponutrin is an important issue that remains to be elucidated. A number of studies already evoke that adiponutrin does not exhibit lipase activity (7–9). Although these findings will need further studies, our results and those of others argue that adiponutrin may favor the anabolic pathway.

Insulin is the principal regulatory hormone involved in the tight regulation of fuel metabolism. A major action of insulin is the regulation of anabolic responses in fat cells, as it stimulates glucose and FFA uptake, inhibits lipolysis, and stimulates *de novo* fatty acid synthesis, through the activation of the insulin-signaling pathway. In clamp studies, mRNA contents of key protein contributors of the insulin action, such as p85 α regulatory subunit of phosphatidylinositol 3-kinase (p85 α PI3-kinase), hexokinase II, the glucose-transporter GLUT4, and the transcription factor sterol regulatory element binding protein-1c (SREBP-1c) also increased after an insulin infusion in adipose tissue of healthy subjects (23). The effect of insulin on these genes has provided arguments for the involvement of the PI3-kinase pathway as a mediator of insulin action together with evidences obtained in cell culture experiments (24–26). Adiponutrin could belong to this class of genes acutely regulated by insulin infusion in human adipose tissue. In type-2 diabetic patients, the regulatory effect of insulin on p85 α PI3Kinase, hexokinase II, GLUT4, and SREBP-1c is altered in the adipose tissue (23) in agreement with an altered PI3-kinase signaling pathway (27–29). GLUT4 and SREBP-1c are also significantly decreased in the adipose tissue of obese non-diabetic subjects (23). Adiponutrin showed a different pattern with a reduction of its basal expression in the adipose tissue of type-2 diabetic patients, but not in obese subjects, and an induction upon insulin

infusion in type-2 diabetic patients. These data also suggest that adiponutrin gene dysregulation may be associated with impaired insulin sensitivity and also a defective glucose tolerance in adipose tissue as observed in type-2 diabetics, but not in obese patients.

The specific molecular mechanisms regulating the adiponutrin gene expression in human adipose tissue are unknown. Tracks might be provided by future studies of transcription factors targeted by insulin. Indeed, the adiponutrin gene exhibits features of lipogenic genes (3), being mainly regulated by the SREBP-1c factor in adipose tissue (30–32). The new transcription factor, carbohydrate responsive element binding protein (ChREBP) has been suggested to mediate glucose action in liver. In adipose tissue, its expression is subjected to combined regulation by glucose and insulin (33, 34). Noteworthy computer analysis has provided evidence for the presence of sterol response element and carbohydrate response element consensus sites in the mouse adiponutrin promoter (unpublished observation), suggesting that both SREBP-1c and ChREBP could transactivate the adiponutrin promoter. Some degree of regulation by peroxisome proliferator-activated receptor γ has also been suggested since it was reported that adiponutrin gene expression is suppressed by troglitazone in 3T3-L1 adipocytes (35). Molecular studies are needed to identify the molecular mechanisms involved in the regulation at the level of gene and protein expression.

In conclusion, the adiponutrin gene is strongly and rapidly regulated by insulin and/or glucose in humans. Although the physiological role of this protein is not established, *in vivo* and *in vitro* results suggest that adiponutrin may participate more likely in the anabolic pathway in adipose tissue. Further investigations are needed to clarify the function of this new protein in the modulation of whole body glucose homeostasis.

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