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Expression of subcutaneous adipose tissue phosphoenolpyruvate carboxykinase correlates with body mass index in nondiabetic women

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Abstract

Phosphoenolpyruvate carboxykinase (*PEPCK*) is a key enzyme for glyceroneogenesis in adipose tissues. Dysregulated glyceroneogenesis is associated with abnormal fatty acid homeostasis, obesity, and insulin resistance in both animal and cellular studies. However, the role of *PEPCK* expression in human adipose tissues on metabolic phenotypes has not been explored. This study aimed to analyze the correlation between *PEPCK* messenger RNA (mRNA) expressions in the subcutaneous adipose tissues with obesity-related metabolic phenotypes. We obtained the demographic data, biochemical variables, and abdominal subcutaneous adipose tissue from 75 nondiabetic nonmenopausal women. The relative *PEPCK* mRNA levels were quantified by real-time polymerase chain reaction normalized with β-actin as a control. The *PEPCK* mRNA levels of subcutaneous tissue were positively correlated with body mass index (BMI) using either univariate ($r = 0.413$, $P < .001$) or multivariate linear regression analysis ($\beta = .978 \pm .239$, $P < .001$). The mRNA expression of *PEPCK* was also positively correlated with body fat percentage ($r = 0.436$, $P < .001$), plasma triacylglycerol, and total cholesterol levels (both P values $< .001$). However, the significant correlation between lipid profile and *PEPCK* expression in subcutaneous tissue was abolished after adjusting for BMI. The relative subcutaneous *PEPCK* mRNA level was not correlated with fasting plasma glucose and insulin, and with an insulin resistance index measured with homeostasis model assessment. In conclusion, we showed that *PEPCK* mRNA expression in the subcutaneous adipose tissues was associated with BMI and plasma triacylglycerol and total cholesterol levels, but was not correlated with insulin resistance index.

1. Introduction

Adipose tissue is not only a depot for energy storage but also an active endocrine organ [1,2]. Many biologically active factors, including cytokines, peptides, and fatty acids, secreted from adipose tissue may produce profound effects on the adipose tissue itself as well as on the other tissues of our body [1,2]. Many genes expressed in the adipose tissue, such as peroxisome proliferators–activated receptor γ (PPARγ), leptin, adiponectin, and tumor necrosis factor α, play an important role in insulin sensitivity, glucose disposal, and fatty acid metabolism [1,2].

Increased concentration of nonesterified fatty acids (NEFAs) in the blood is an early finding in patients with type 2 diabetes mellitus [3]. Short-term elevation of plasma NEFAs by short-term infusion causes insulin resistance by interfering with the insulin transduction cascade [4,5]. Concentrations of NEFAs in the bloodstream come from a complex interplay between hydrolysis of triacylglycerol (TG) and reesterification of the NEFAs with glycerol 3-phosphate in the fat cells. It was demonstrated that a significant part of NEFA (30%-70%) is reesterified, so that a recycling occurs and net fatty acid output is much less than true lipolysis [6]. Therefore, alterations in the processes of fatty acid storage and increased release of NEFA-form adipose tissue can cause insulin resistance [7]. In adipose tissue, glucose is considered to be the main precursor of glycerol 3-phosphate. When the supply of glucose is limited, as seen in starvation or on a low-carbohydrate diet, glyceroneogenesis originating from lactate, pyruvate, or

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specific amino acids occurs [8-10]. The key enzyme involved in glyceroneogenesis is cytosolic phosphoenolpyruvate carboxykinase (*PEPCK*) [11]. This was confirmed later by inhibiting or enhancing the expression of *PEPCK* in adipose tissues obtained from different transgenic mice models [12,13]. There is no known posttranslational modification of this enzyme, and the changes in *PEPCK* messenger RNA (mRNA) are directly related to the enzymatic activities in the tissues [14,15].

The glyceroneogenesis pathway has been ignored for many years. Recently, Beale et al [16] reported that glyceroneogenesis is indeed important to lipid homeostasis and that a deregulation in this pathway may have profound pathophysiological effects and ultimately lead to type 2 diabetes mellitus. There is some evidence supporting the hypothesis. Thiazolidinediones (TZDs) blocked NEFA release by inducing glyceroneogenesis in fat cells [17], and the mRNA expression of *PEPCK* gene in fat cells is also strongly induced by TZDs via PPAR response element binding and transactivation in adipose tissue at the transcriptional level [18]. The role of glyceroneogenesis to human disease has been further investigated by pathophysiological studies and linkage analyses. It has been reported that cytosolic *PEPCK* mRNA was induced either in white adipose tissue of TZD-treated type 2 diabetes mellitus humans [19] or in TZD-treated white adipose tissue explants [20]. Genetic linkage studies suggest that a susceptibility locus to type 2 diabetes mellitus maps to the region containing the promoter sites of *PEPCK* gene on human chromosome 20q13 [21-23]. Because there is a clustering of unfavorable body fat distribution, glucose intolerance or type 2 diabetes mellitus, hyperinsulinemia, and hypertriglyceridemia in subjects with the metabolic syndrome [24,25], we therefore studied the relation between cytosolic *PEPCK* mRNA expression in the subcutaneous adipose depot and several metabolic phenotypes including serum level of insulin, TG, fasting plasma glucose (FPG), degree of insulin sensitivity, and body mass index (BMI) in a group of nondiabetic subjects.

2. Methods

2.1. Subjects

We recruited 75 nondiabetic (according to the American Diabetes Association criteria 1997) nonmenopausal female subjects, aged 19 to 54 years. The abdominal subcutaneous adipose tissues were obtained during a scheduled elective surgery either for benign uterine myoma or for a gastric partition laparoscopic surgery for obesity at a fasting state. Among them, 30 subjects fulfilled the criteria of morbid obesity [26]. Informed consent was obtained from each patient. This study was approved by the institutional review boards.

Blood samples were taken on the next morning after their admission to the hospital before operation. The FPG, serum insulin, and TG were measured according to previous reports [27-29]. Insulin resistance index was calculated with the homeostasis model assessment (HOMA-IR) as described previously [30]. Body fat percentage was calculated with the following formula: $1.2 \times \text{BMI} + 0.23 \times \text{age} - 10.8 \times \text{sex} - 5.4$ (male = 1, female = 0) [31].

2.2. Adipose tissue RNA extraction and reverse transcription

The adipose tissue was immediately dipped into liquid nitrogen after removal and then stored in a freezer at −80°C until use. Total RNA was extracted using Rezol (Promega, Madison, WI) following manufacturer's recommendation. Reverse transcription was performed with 1 μg of total RNA and 0.5 μg of random hexamers in a final volume of 25 μL containing 200 U of Maloney murine leukemia virus reverse transcriptase, 20 nmol/L deoxynucleotide triphosphate, and 25 U of rRNasin for 1 hour at 37°C using a reverse transcription kit (Promega). The complementary DNA (cDNA) products were diluted to 100 μL with distilled deionized water before polymerase chain reaction (PCR) for amplification.

2.3. Quantitation of mRNA by real-time PCR

Two microliters of diluted cDNA was added to a 12.5-μL 2× SYBR Green PCR Master Mix (Perkin-Elmer Applied Biosystems, Foster City, CA), variable amounts of the respective primers for the human cytosolic *PEPCK* and β-actin, and water to a final volume of 25 μL. The primers for *PEPCK* (forward: 5′-TAT GAC AAC TGC TGG TTG GC-3′ and reverse: 5′-ATA ACC GTC TTG CTT TCG ATC-3′) and β-actin (forward: 5′-CCT CAT GAA GAT CCT CAC CAC CGA GC-3′ and reverse: 5′-GCC AAT GGT GAT GAC CTG GC-3′) were designed with PRIMER Express software (Perkin-Elmer Applied Biosystems). The PCR conditions were 10 minutes at 95°C and 40 cycles of 15 seconds at 95°C plus 1 minute at 60°C. Detailed principle and procedures for the real-time quantitative PCR were according to the users' bulletin and manual of ABI PRISM 5700 Sequence Detection System (Perkin-Elmer Applied Biosystems). In brief, the fluorescent signal from each PCR reaction is collected as the peak normalized values plotted vs the cycle numbers. Reactions are characterized by comparing the *threshold cycle number* (Ct), a value defined as the fractional cycle number at which the normalized sample fluorescence signal exceeds a fixed threshold above baseline when it is always located within the linear phase of amplification. Samples with a high starting copy number of cDNA show an increase in fluorescence earlier in the PCR process, thus resulting in a low Ct number.

The comparative Ct method is to display target gene expression (*PEPCK* in this study) relative to that of endogenous control gene (β-actin in this study), that is, ΔCt = Ct number of β-actin − Ct number of *PEPCK*. The ΔCt indicates a $\log_2$ transformation of the *PEPCK* mRNA expression relative to that of β-actin. This method eliminates

the need for standard curve. Therefore, if the expression level of β-actin is higher than that of *PEPCK* gene in the same cDNA sample, the result of ΔCt (Ct number of β-actin − Ct number of *PEPCK*) will be negative. In contrast, if the expression level of *PEPCK* gene is higher than that of β-actin, the result of ΔCt will be positive. In other words, the higher the ΔCt level, the higher the *PEPCK* gene expression level relative to β-actin expression.

2.4. Statistical analysis

Results were expressed as means ± standard error (SE). Pearson correlation was used to examine the relation between the mRNA levels and the various metabolic variables. Multivariate linear regression analyses were performed using BMI, glucose, insulin, HOMA-IR, TG, total cholesterol, and the relative mRNA level of *PEPCK* also in different models as indicated. The statistical analyses were performed using SPSS 10.0 version (SPSS, Chicago, IL). A *P* value of less than .05 was considered statistically significant.

3. Results

3.1. Demographic and anthropometric characteristics

Demographic and anthropometric characteristics of the study subjects were listed in Table 1. There are 75 nondiabetic nonmenopausal women included in this study, and the subjects in this study are relatively obese but with a wide range of BMI from 17.3 to 48.6 kg/m^2.

3.2. Relative PEPCK mRNA levels of subcutaneous adipose tissue were significantly correlated with BMI, cholesterol, and TG, but not insulin resistance index

In simple linear regression analyses, the relative *PEPCK* mRNA levels from subcutaneous tissues were significantly correlated with BMI ($r = 0.413$, $P < .001$, Fig. 1), body fat percentage ($r = 0.436$, $P < .001$), plasma TG concentrations ($r = 0.400$, $P < .001$), and plasma total cholesterol concentrations ($r = 0.325$, $P = .004$). In contrast, the subcutaneous *PEPCK* mRNA expression was not correlated with FPG ($r = 0.08$, $P = .486$), insulin ($r = 0.093$, $P = .486$), and HOMA-IR ($r = 0.102$, $P = .445$) (data not shown). On the other hand, BMI was significantly correlated with TG ($r = 0.459$, $P < .001$), cholesterol ($r = 0.500$, $P < .001$), log(plasma insulin) ($r = 0.303$, $P = .004$), and log(HOMA-IR) ($r = 0.311$, $P = .003$).

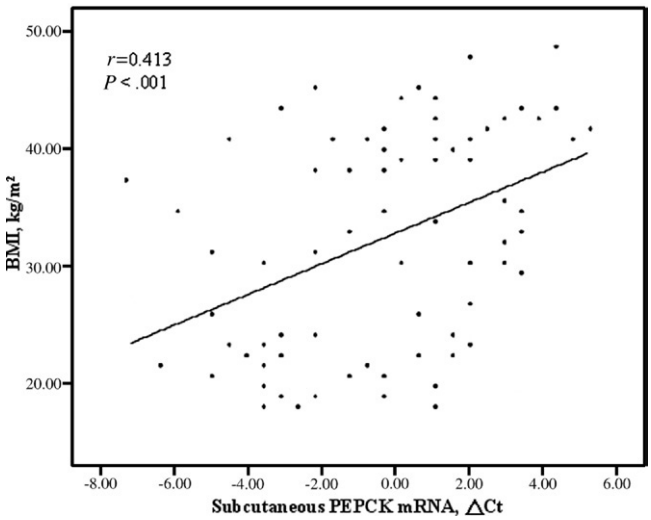

Fig. 1. The correlation between the relative PEPCK mRNA levels in the subcutaneous adipose depot and BMI among 75 nondiabetic nonmenopausal women.

3.3. Subcutaneous PEPCK expression levels were independently correlated with BMI after adjusting for age

Because BMI was positively correlated with both plasma TG and total cholesterol levels, we then performed multivariate linear regression analyses to adjust for the potential confounding effect of age and BMI. Firstly, we adjusted for the effect of age in various regression models. As shown in Table 2, we found that BMI was significantly correlated with age and relative subcutaneous *PEPCK* mRNA level, suggesting that *PEPCK* mRNA expression is independently associated with BMI even after adjusting for age. We then analyzed the relation of subcutaneous *PEPCK* mRNA expression and blood lipids (Tables 3 and 4). Plasma TG and cholesterol levels were significantly correlated with subcutaneous *PEPCK* mRNA levels while adjusting for age. However, the significance was abolished when further adjusted for BMI in the regression models (model II in Tables 3 and 4), indicating that the correlation between subcutaneous *PEPCK* mRNA levels and blood lipid levels is in part mediated by the effect of BMI. Body mass index presumably causes the elevations both in blood lipids and *PEPCK* mRNA levels.

Table 1
The demographic and biochemical characteristics of 75 nondiabetic women

Variables	Mean ± SE	Ranges
Age (y)	36.9 ± 1.1	18.0-54.9
BMI (kg/m^2)	32.4 ± 1.1	17.3-48.6
Body fat percentage (%)	42.0 ± 1.1	25.4-59.6
Glucose (mmol/L)	5.4 ± 0.1	4.3-6.9
Insulin (pmol/L)	131.1 ± 12.6	8.6-603.4
Triglycerides (mmol/L)	1.5 ± 0.1	0.3-5.4
Total cholesterol (mmol/L)	4.9 ± 0.1	3.0-7.2

Table 2
Multivariate linear regression using BMI as dependent variable

Independent variables	Regression coefficients ± SE	95% CI of regression coefficients	*P*
Age	−0.634 ± 0.075	−0.781 to −0.487	<.001
Subcutaneous *PEPCK*, ΔCt	0.978 ± 0.239	0.510 to 1.446	<.001

Table 3
Multivariate linear regression using TG as dependent variable

	Regression coefficients ± SE	95% CI of regression coefficients	*P*
Model I			
Age	−0.018 ± 0.011	−0.040 to 0.004	.1
Subcutaneous *PEPCK*, ΔCt	0.122 ± 0.035	0.053 to 0.191	.001
Model II			
Age	0.021 ± 0.014	−0.006 to 0.048	.131
Subcutaneous *PEPCK*, ΔCt	0.062 ± 0.035	−0.007 to 0.131	.08
BMI	0.061 ± 0.015	0.032 to 0.090	<.001

Fasting plasma glucose, insulin, and HOMA-IR levels were not correlated with the *PEPCK* mRNA expression levels when adjusted for various variables (data not shown).

3.4. Subcutaneous PEPCK expression levels were significantly different among lean, overweight, and obese groups

The 75 subjects were assigned to lean (BMI 17-25, n = 26), overweight (BMI 26-30, n = 5), or obese (BMI ≥ 30, n = 44) group. It showed that the higher the BMI, the higher the relative *PEPCK* expression in subcutaneous adipose tissue (lean, −1.87 ± 0.46; overweight, 0.24 ± 1.38; obese, 0.52 ± 0.44). There was significant difference of relative *PEPCK* expression in subcutaneous adipose tissue between lean and obese groups (mean difference ± SE, −2.39 ± 0.67; 95% confidence interval [CI], −3.72 to −1.05; *P* value = .001). There was borderline significant difference between lean and overweight groups due to small sample size (mean difference ± SE, −2.11 ± 1.21; 95% CI, −4.59 to 0.36; *P* value = .092). There was no significant difference between overweight and obese groups (mean difference ± SE, −0.27 ± 1.39; 95% CI, −3.06 to 2.51; *P* value = .844).

4. Discussion

It has been demonstrated that protein level and the enzymatic activity of *PEPCK* are tightly regulated at the pretranslational level [14,15]. It is therefore plausible to study the biological function of the *PEPCK* gene by measuring mRNA with quantitative PCR as shown in our present study. We found that *PEPCK* mRNA expression level in subcutaneous adipose tissue was positively correlated with BMI, body fat percentage, fasting TG, and total cholesterol levels in nondiabetic nonmenopausal women.

Insulin resistance and type 2 diabetes mellitus are in part due to the deregulated fatty acid metabolism that leads to a high plasma NEFA concentration [32]. The function of *PEPCK* in the adipose tissue has been demonstrated to be responsible for glyceroneogenesis, by which the newly synthesized glycerol combines with fatty acids to form TG catalyzed by the glycerol kinase. Glyceroneogenesis takes place in the fasting state, and the substrates (lactate, pyruvate, or specific amino acids) are also as important as the *PEPCK* enzyme in fueling the reaction [8-10]. Underexpression of the *PEPCK* expression might impair this process and result in the release of NEFAs into the bloodstream, thus causing lipodystrophy and insulin resistance [12]. By contrast, the transgenic mice with overexpression of *PEPCK* in adipose tissues develop obesity as a result of increased glyceroneogenesis and fatty acid reesterification [13]. Despite increased fat mass, a study in these transgenic mice showed that circulating NEFA was decreased and glucose tolerance and whole-body insulin sensitivity were completely normal [13]. However, when the transgenic mice were fed with high-fat diet, they developed severe obesity and became more hyperinsulinemic, glucose intolerant, and insulin resistant than control mice [33]. Moreover, they displayed higher levels of circulating triglycerides associated with a higher degree of hepatic steatosis. The high triglyceride accumulation prevented white adipose tissue from buffering the flux of lipids in circulation and led to increased serum triglyceride levels and fat deposition in liver [33]. These results indicate that increased *PEPCK* expression in the presence of high-fat feeding may have deleterious effects and lead to severe insulin resistance and type 2 diabetes mellitus. In our human study, we found that the subcutaneous *PEPCK* mRNA levels were positively correlated with BMI but not with insulin resistance measured with HOMA. Although we did not measure plasma NEFA levels, our data were consistent with studies in the transgenic mice under normal diet [13]. Moreover, after adjustment for age, the correlation between subcutaneous *PEPCK* mRNA levels and BMI remained significant.

Plasma TG and total cholesterol levels were also found to be correlated with the subcutaneous *PEPCK* mRNA levels. Because the blood lipid levels are highly correlated with BMI, our observations that the significant correlation between plasma TG and total cholesterol levels was reduced when BMI was taken into consideration (model II in Tables 3 and 4) indicate that the correlation between subcutaneous *PEPCK* mRNA expression and blood lipids level is explained by the presence of obesity.

Table 4
Multivariate linear regression using total cholesterol as dependent variable

	Regression coefficients ± SE	95% CI of regression coefficients	*P*
Model I			
Age	−0.029 ± 0.011	−0.051 to −0.007	.010
Subcutaneous *PEPCK*, ΔCt	0.094 ± 0.035	0.025 to 0.163	.010
Model II			
Age	0.019 ± 0.013	−0.006 to 0.044	.159
Subcutaneous *PEPCK*, ΔCt	0.019 ± 0.034	−0.048 to 0.086	.574
BMI	0.076 ± 0.015	0.047 to 0.105	<.001

In subjects with type 2 diabetes mellitus, administration of TZD, the PPARγ agonist, improves glucose tolerance together with an increase in body weight and BMI to a certain extent [34]. Detailed study by using computerized tomography scan revealed that treatment with TZD causes body fat redistribution, that is, a decrease of visceral fat and an increase of subcutaneous fat [35]. The mechanism of TZD-related redistribution of fat accumulation is not well understood. Some studies suggest that *PEPCK* gene expression might mediate this response because TZD might increase *PEPCK* gene expression via an adipose tissue–specific PPARγ response element in its promoter region [7,18]. Interestingly, a region near the *PEPCK* locus on chromosome 20 has been mapped for human obesity in genetic linkage study [35]. Whether this important pathway serves as a therapeutic avenue for obesity remains to be investigated.

There are certain limitations of this study. For examples, because this observation was only made in nondiabetic nonmenopausal women, the generalization to the whole population remains unknown. Moreover, the measurement of insulin sensitivity is indirect and might not be sensitive enough for detecting a small effect of change in whole-body insulin sensitivity. Finally, we did not measure FFA levels, although a basal level of FFA is not a good indicator for insulin sensitivity (Chuang et al, unpublished data).

In summary, we performed a human study to correlate the expression of *PEPCK* gene in the subcutaneous adipose depot with different metabolic phenotypes. We found that expression of *PEPCK* gene correlates with BMI independent of age in women. The correlations between *PEPCK* mRNA and plasma TG and cholesterol levels were dependent of BMI. Whether the control of *PEPCK* gene expression plays a primary role in human obesity needs to be further confirmed.

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