Effects of calorie intake and sampling time on thyroid stimulating hormone concentration

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Research Article

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Abstract

Background

This study aimed to investigate the effects of blood collected after calorie intake on the level of thyroid stimulating hormone (TSH), comparing with the blood collected in fasting state.

Methods

This study was a prospective, randomized, controlled study. Subjects from the outpatients in the department of endocrinology without evident of thyroid diseases were included and then randomized into the fasting group, diet intake group, and glucose intake group, respectively. Fasting blood was collected from all the subjects at 7:00 am for the measurement of TSH and free thyroxine (FT4). Afterward, the subjects were maintained at fasting state (fasting group), had an intake of the mixed diet with the calories of 400 Kcal (diet intake group), and had an intake of 75 g glucose (glucose intake group), respectively, and blood was acquired again 2 h later (9:00 am on the same day) for TSH and FT4 measurement. The levels between 7:00 am and 9:00 am were compared.

Results

Of the 150 subjects, 146 met the inclusion criteria, of which 48, 48, and 50 were in the diet intake group, glucose intake group, and fasting group, respectively. The TSH in the diet intake group was significantly lower at 9:00 am (TSH_{9am}) than the level at 2h before (TSH_{7am}) (*P<0.001*), of which the median variation was -0.71 mU/L, and the median variation rate was -32.4%. In the glucose intake group, the TSH_{9am} was also significantly lower than TSH_{7am} (*P<0.001*), of which the median variation was -0.73 mU/L, and median variation rate was -31.5%. While in the fasting group, the TSH_{9am} decreased slightly but statistically significantly lower than TSH_{7am} (*P<0.001*), of which the median variation was -0.1 mU/L, and the median variation rate was -5.2%. According to TSH_{7am}, 9 subjects in total (3 subjects in each group) met the diagnostic criteria of subclinical hypothyroidism. However, according toTSH_{9am}, only 2 patients in the fasting group still met the diagnostic criteria of subclinical hypothyroidism.

Conclusion

Comparing with the fasting state, the TSH level at 2h after the calorie intake was decreased by about 30%, which could influence the diagnosis of subclinical hypothyroidism.

Trial registration

ChiCTR2100047454.

Background

Thyroid-stimulating hormone (TSH) is synthesized by the anterior pituitary gland, which regulates the synthesis and secretion of thyroid hormones, and is also regulated by the feedback of thyroid hormones [1]. Guidelines of American and European thyroid associations [2, 3] have recommended using TSH level as the indicator to screen the thyroid dysfunctions. The earliest change in patients with primary thyroid dysfunctions is TSH level alteration. For instance, patients with subclinical thyroid dysfunctions are only with TSH level changes, characterized TSH elevation in subclinical hypothyroidism and TSH reduction in subclinical hyperthyroidism. Subclinical hypothyroidism is associated with dyslipidemia, hypertension, metabolic syndrome [4], adverse maternal-fetal outcomes [2], and possibly cardiovascular diseases [5]. The TSH is also the major indicator for deciding whether treatments should be initiated in patients with subclinical hypothyroidism, as well as for evaluating whether the intervention target has been achieved. Therefore, accurately measuring TSH levels is critical and of clinical significance.

Previous studies have reported that TSH secretion and thus the blood level has a circadian rhythm [6–11]. The relative amplitude (maximum absolute value of a periodically varying quantity) of TSH within one day could be as high as 36%. The variation of TSH during daytime is relatively small, but the food intake could influence the TSH level [12–17]. However, there are no special recommendations on the time of blood sampling for TSH measurement, as well as the requirement of fasting in the guidelines [2, 3]. In addition, the sampling time, and the fasting state for evaluating the reference range of thyroid hormone by laboratories are generally neglected in clinical practices. Analyzing the data from clinical laboratories showed that blood samples were delivered from 7:00 am to 4:00 pm for TSH measurement, and the distribution of TSH levels measured at different times in patients differed significantly [18]. Our previous pilot studies (data not published) showed that the TSH level measured at 2 h after the meal (9:00 am), comparing with the fasting blood samples collected in the morning (7:00 am) was significantly reduced by about 30%, and there was a significant difference in the diagnosis of subclinical hypothyroidism between the two sampling methods.

Due to the circadian rhythm of the blood TSH levels, it is necessary to further investigate the difference of the blood TSH level at 7:00 am and 9:00 am after meal to determine the influence by the intake of food or diurnal rhythm. In addition, we aimed to evaluate the influence of different food components on blood TSH levels. Therefore, this study was to investigate the trend of the blood TSH variation in subjects maintained in the fasting state or after the intake of calories contributed from different nutrients, comparing with the fasting state in the morning.

Methods

Subjects

The subjects were selected from the outpatients in the Department of Endocrinology at the Peking University First Hospital between February 2021 and March 2021. The inclusion criteria were as follows: 1) patients aged 20-80 years; 2) subjects without an evident history of thyroid diseases; and 3) patients whose physical examinations showed no sign of thyroid enlargement. The exclusion criteria were as

follows: 1) patients with acute infectious diseases; 2) subjects with abnormal liver functions with elevated levels of ALT and/AST to higher than 2.5 folds of the upper limit of reference range; 3) patients with chronic renal diseases with the serum creatinine level of >130 μmol/L; 4) subjects treated with glucocorticoids, contraceptive pills, or other hormones; 5) subjects with the history of hypothalamic-pituitary diseases; 6) pregnant women. The study was approved by the Ethics Committee of Peking University First Hospital, and all the participants signed informed consent. The registration number of the study was ChiCTR2100047454.

Randomization of subjects

SPSS statistics were used to generate random numbers (random number seeds: 20000000), which was used for 1:1:1 randomization of the subjects into 3 groups, namely diet intake group, glucose intake group, and fasting group.

Interventions

For all the subjects who participated in this study, the first blood sample was collected after fasting for over 10 h (about 7:00~7:30 am). Afterward, subjects in the diet intake group were asked to intake mixed diets (with the calories of about 400 Kcal), the time was recorded from the first bite of food, and the blood was collected again 2 h later (about 9:00~9:30 am). Subjects in the glucose intake group were asked to intake 75 g glucose (calories of 300 Kcal), the time was recorded from the first sip of glucose solution, and the blood was collected again 2 h later (about 9:00~9:30 am). Subjects in the fasting group maintained a fasting state, and the blood was collected again 2 h later (about 9:00~9:30 am).

Endpoints

The variation of TSH after fasting for 2 h or at 2 h after calorie intake, comparing with the baseline level at 7:00 am, as well as the influence of blood collection time on the diagnosis of thyroid dysfunctions were the endpoints of this study.

Measurement protocols

The blood serum samples were stored at -20°C, and the TSH and FT_4 were measured in the same sample. The TSH and FT_4 levels were measured by the acridinium ester chemiluminescence assay using the Siemens Centaur XP machine. The sensitivity of measuring TSH was 0.019 mU/L, the intra-batch variable coefficient was 1.98%, and the reference range was 0.55-4.78 mU/L. The sensitivity of measuring FT_4 was 1.3 pmol/L, the intra-batch variable coefficient was 1.95%, and the reference range was 11.48-22.70 pmol/L.

Statistical methods

Sample size estimation: The finding of the pilot study showed that the reduction rate was 30% in the diet intake group and glucose intake group and 7% in the fasting group. The online tool http://powerandsamplesize.com/Calculators/ was utilized to calculate the sample size. The one-way-ANOVA-pairwise mode for the comparison of means among multiple groups was used for the sample

size estimation, with the β = 0.1, α = 0.05, and the number of groups of 3. The findings showed that 46 subjects were required for each group. Therefore, 50 subjects were planned to be included in each group in this study.

The SPSS 24.0 software (IBM, Armonk, NY, USA) was used for data processing and statistical analysis. Normality test by the Kolmogorov test was performed for continuous data; the continuous data in normal distribution were described with means ± standard divisions, while continuous data that were not in normal distribution were described with medians (interquartile ranges). For the comparison of the levels between two time points of blood sampling within the same group, paired t-test was used for the data in the normal distribution, and paired Wilcoxon signed-rank test was used for the data in the abnormal distribution. For the comparisons among 3 groups, one-way analysis of variances (one-way ANOVA) followed by post-hoc Bonferroni test was used for the data in the normal distribution, and Kruskal-Wallis one-way ANOVA was used for data that were not in the normal distribution, with the Bonferroni test used to correct the significance for pair-wise comparisons. Linear regression was performed using TSH variation amplitude as the dependent factor. $P<0.05$ (two-sided) was considered statistically significant. The GraphPad Prime 7.04 software was used for plotting the figures.

Results

General characteristics of the subjects

A total of 150 subjects participated in this study, of which 4 were excluded for the serum creatinine level of >130 μmol/L. Therefore, finally, 146 subjects including 70 males and 76 females, met the inclusion criteria. Forty-eight, 48, and 50 of the subjects were randomly included in the diet intake group, glucose intake group, and fasting group, respectively. The sex, age, liver function, and renal function of the subjects in the 3 groups were not significantly different (Table 1).

Table 1
General characteristics and thyroid function of the subjects in the 3 groups at 7:00 am

	Diet intake group	Glucose intake group	Fasting group	Total
n	48	48	50	146
Sex (M/F)	25/23	20/28	25/25	70/76
Age (Years)	60.0 (51.0, 65.0)	59.0 (50.0, 64.3)	59.0 (49.8, 63.5)	59.0 (51.0, 64.3)
ALT (IU/L)	18.5 (14.0, 31.3)	23.0 (16.0, 27.0)	19.0 (14.0, 26.8)	20.0 (14.0, 27.0)
AST (IU/L)	19.0 (15.0, 32.3)	20.0 (16.0, 23.5)	21.0 (17.0, 23.8)	20.0 (16.0, 25.0)
Serum creatinine (μmol/L)	74.8 (61.9, 81.6)	81 (71, 89.6)	80.4 (64.7, 88.8)	78.1 (65.2, 85.4)
TSH_{7am} (mU/L)	2.3 (1.4, 2.9)	2.2 (1.5, 3.1)	1.9 (1.3, 3.0)	2.2 (1.4, 3)
$FT_{4\ 7am}$ (pmol/L)	16.8±2.0	16.2±2.0	15.8±2.1	16.1±2.1

Data in normal distribution were described with mean±SD, data that were not in normal distribution were described with median (P25, P75).

The baseline TSH level at 7:00 am was 2.3 (1.4, 2.9) mU/L, 2.2 (1.5, 3.1) mU/L, and 1.9 (1.3, 3.0) mU/L in the diet intake group, glucose intake group, and fasting group, respectively, and the differences among the 3 groups were not statistically significant (P=0.628).

TSH level 2 h after fasting or calorie intake

In the diet intake group, the TSH level at 9:00 am (TSH_{9am}) was significantly lower than the level at 2 h before (TSH_{7am}) the fasting state (TSH_{7am} vs TSH_{9am}: 2.3 (1.4, 2.9) vs 1.5 (1.0, 2.1), P<0.001). The median variation amplitude was -0.71 mU/L (95% CI: -0.90, -0.61 mU/L), and the median variation rate was -32.4% (95% CI: -34.6, -27.5%).

In the glucose intake group, the TSH level at 9:00 am (TSH_{9am}) was significantly lower than the level at 2 h before (TSH_{7am}) the fasting state (TSH_{7am} vs TSH_{9am}: 2.2 (1.5, 3.1) vs 1.4 (0.9, 2.1), P<0.001). The median variation amplitude was -0.73 mU/L (95% CI: -1.01, -0.68 mU/L), and the median variation rate was -31.5% (95% CI: -35.8, -30.4%) (Table 2).

Table 2
Influence of fasting and calorie intake on TSH level

Median (P25, P75)	Diet intake group	Glucose intake group	Fasting group	Total
TSH_{7am} (mU/L)	2.3 (1.4, 2.9)	2.2 (1.5, 3.1)	1.9 (1.3, 3)	2.2 (1.4, 3)
TSH_{9am} (mU/L)	1.5 (1, 2.1) ***	1.4 (0.9, 2.1) ***	1.7 (1.2, 3.1) ***	1.6 (1, 2.2) ***
TSH variation amplitude (mU/L)	-0.71 (-1.02, -0.36) ###	-0.73 (-1.05, -0.44) &&&	-0.1 (-0.26, 0.04)	-0.53 (-0.87, -0.16)
TSH variation rate (%)	-32.4 (-39.3, -25.3) ###	-31.5 (-40.7, -24) &&&	-5.2 (-16.1, 1.6)	-27.6 (-37.1, -11.8)

*** P<0.001, comparing with$TSH_{7am;}$ ### P<0.001, comparing with the fasting group; &&& P<0.001, comparing with the fasting group. Data that were not in normal distribution were described with median (P25, P75).

In the fasting group, the TSH level at 9:00 am (TSH_{9am}) was slightly but significantly lower than the level at 2 h before (TSH_{7am}) the fasting state (*P*<0.001). The median variation amplitude was -0.1 mU/L (95% CI: -0.27, 0.05 mU/L), and the median variation rate was -5.2% (95% CI: -11.7, 1.9%). (Table 2)

The variation amplitude and variation rate of the TSH were significantly different among the three groups (*P*<0.001). The pair-wise comparison showed that the TSH variation amplitude and variation rate were not significantly different between the diet intake group and glucose intake group. In contrast, the TSH variation amplitude and variation rate in the fasting group were significantly lower than the diet intake group (*P*<0.001 after Bonferroni correction) and glucose intake group (*P*<0.001 after Bonferroni correction) (Table 2).

Association between TSH variation amplitude and fasting TSH_{7am} level

The TSH variation amplitude was significantly negatively correlated with the TSH_{7am} level (Spearman rank correlation coefficient: -0.551 for all subjects, -0.809 for diet intake group, -0.881 in glucose intake group, and -0.135 for the fasting group).

Single-factor linear regression was performed using the TSH_{7am} as the independent factor, and the TSH variation amplitude as the dependent factor (Figure <link rid="fig1">1</link>-1). The regression coefficient and determination coefficient (R^2) were -0.329 (95% CI: -0.402, -0.255, *P*<0.001) and 0.637 in the diet intake group, -0.375 (95% CI: -0.417, -0.311, *P*<0.001) and 0.835 in the glucose intake group, and -0.073 (95% CI: -0.124, 0.337, P=0.223) and 0.026 in the fasting group, respectively.

The comparison between the diet intake group and glucose intake group showed that the TSH variation amplitude was not significantly different after calorie intake. The linear regression using the fasting TSH as the independent factor also showed that the regression coefficient and intercept were not significantly different between the two groups. Therefore, data of these two groups were combined as the calorie intake group, and then linear regression analysis was performed for TSH variation amplitude and TSH_{7am} (Figures 1-2), which showed the regression coefficient was -0.357 (95% CI: -0.402, -0.255, P<0.001), and R^2 was 0.748. These findings suggested that 74% of the TSH variation amplitude was determined by the TSH_{7am}.

The partial correlation analysis with TSH_{7am} controlled showed that TSH variation amplitude was not significantly correlated with age and fasting FT_4.

Influence of blood sample collection at different times on the evaluation of thyroid functions

According to the TSH and FT_4 levels measured at 7:00 am and 9:00 am, no subject included in this study was with clinical thyroid dysfunction.

Subjects with TSH of >4.78 mU/L and FT_4 level in the reference range were diagnosed with subclinical hypothyroidism. According to the levels measured at 7:00 am in the fasting state, 3 subjects in each group met the diagnostic criteria of subclinical hypothyroidism. However, according to the TSH level measured at 9:00 am that maintaining fasting or after calorie intake, the number of subjects diagnosed with subclinical hypothyroidism decreased. Specifically, no subject in the diet intake group or glucose intake group was diagnosed with subclinical hypothyroidism, and only 2 subjects in the fasting group were diagnosed with subclinical hypothyroidism.

Subjects with TSH of <0.55 mU/L and FT_4 level in the reference range were diagnosed with subclinical hyperthyroidism. According to the levels measured at 7:00 am in the fasting state, 1, 1, and 0 subjects in the diet intake group, glucose intake group, and fasting group met the diagnostic criteria of subclinical hyperthyroidism, respectively. However, according to the TSH level measured at 9:00 am that maintaining fasting or after calorie intake, the number of subjects diagnosed with subclinical hyperthyroidism increased. Specifically, 1, 2, and 1 subjects in the diet intake group, glucose intake group, and fasting group met the diagnostic criteria of subclinical hyperthyroidism, respectively (Table 3).

Table 3
Number of subjects with subclinical thyroid dysfunctions according to the measurements at different time points

N (%)		Diet intake group	Glucose intake group	Fasting group	Total
Subclinical hypothyroidism	7 am (fasting state)	3 (6.3%)	3 (6.3%)	3 (6.0%)	9 (6.2%)
	9 am (2 h after fasting or calorie intake)	0 (0%)	0 (0%)	2 (4.0%)	2 (1.4%)
Subclinical hyperthyroidism	7 am (fasting state)	1 (2.1%)	1 (2.1%)	0 (0%)	2 (1.4%)
	9 am (2 h after maintained fasting or calorie intake)	1 (2.1%)	2 (4.2%)	1 (2.0%)	4 (2.7%)

Discussion

The findings of this study showed that the TSH level was reduced significantly by about 30% after calorie intake in the morning, The components of calories had no significant influence on TSH variation rate when the calories intake was similar. The TSH level reduced slightly by 5.2% in the subjects that maintained the fasting. The rate of TSH reduction was significantly pronounced after the calorie intake than the fasting state, suggesting that the influence of food on TSH was more evident than the diurnal rhythm of the TSH. Blood sample collection after the calorie intake could significantly influence the diagnosis of subclinical thyroid dysfunction, especially subclinical hypothyroidism.

Previous studies have reported that the TSH level has an apparent circadian rhythm [6–11], during which the level peaks at 2-4 am, and reaches the lowest level at 3-8 pm. With the elapse of time in the morning, the TSH level tends to reduce. The early studies on the rhythm of TSH showed no influence of food intake on TSH level [10]. However, the subsequent studies showed that food intake could influence the level of TSH measurements [12–17, 19], and the rate of TSH reduction was 10-35%.

Many studies only focused on the variation of TSH before and after food intake, but TSH is also influenced by circadian rhythm, and time-lapse could also influence the TSH level. However, only very few studies investigated whether food intake could influence TSH level independent of diurnal rhythm, and the findings were controversial. One study [14] of 20 subjects with normal thyroid functions showed that the serum TSH level at 60 min after lunch and supper intake (calorie of 1061 Kcal) was significantly lower than before the diet, and the reduction was more pronounced after having lunch (median: -0.25 mU/L) than supper (-0.2 mU/L). The reduction amplitude of TSH was -0.1 mU/L after having low-calorie food for lunch (212 Kcal), which was lower than having high-calorie food (1061 Kcal; reduction amplitude: -0.25 mU/L). These findings demonstrated that food intake could independently reduce the level of TSH, which was agreeable with our findings. However, the study did not investigate the influence of breakfast intake on the TSH level. In the present study, the TSH reduction was more pronounced after

the breakfast intake (median value about -0.7 mU/L), which could be associated with the variation of TSH at different times due to diurnal rhythm. The TSH level tends to reduce in the morning, which is enhanced by the influence of the food intake; while the TSH level in the afternoon tends to increase, which could alleviate the influence of food intake. However, another study [17] showed that compared with the fasting state at 7-8 am, the amplitude of TSH reduction at 140 min after food intake of their own choices was similar to fasting for 140 min (-29.3% vs -28.3%). The differences in the findings could be associated with the differences in the time of blood sample collection, the time between the two blood samplings, and calorie intake from food.

In light of the findings of previous studies, we speculated that the TSH reduction after breakfast observed in this study could be from the combined influences by the food intake and time (the diurnal rhythm of TSH). However, the influence of food intake seemed to be more prominent. The causes of the food intake on TSH, are still unclear yet. We speculated that the reduction of TSH could be associated with the acute elevation of somatostatin level after the food intake [20], as somatostatin could inhibit the synthesis and secretion of TSH [1, 11].

The findings of this study showed that the variation of TSH level after calorie intake in the morning significantly influenced the diagnosis of subclinical thyroid dysfunction. Subjects with subclinical hypothyroidism could be underestimated due to the non-fasting state, while the subjects with subclinical hyperthyroidism could be overestimated. In certain conditions, such as pregnancy, the ideal range of TSH is narrowed down, and the TSH value is required to decide the treatment strategy, while the variations of the TSH level could lead to significant clinical influences.

The limitations of this study could be as follows: all the subjects in this study were outpatients and from the Department of Endocrinology of only one hospital, and the subjects were with underlying diseases, such as diabetes, hypertension, and osteoporosis. The findings need to be further validated by future studies with higher representativeness, especially in subjects with a relatively narrow range of the TSH level, such as pregnant women. Furthermore, subjects in this study only consumed one level of calories, and the influences of food of different calories were not investigated.

Conclusion

In summary, the TSH level was reduced significantly after the food intake, comparing with the level at fasting state in the morning. The variation of the TSH level could influence the diagnosis of subclinical thyroid dysfunction. If the reference range of TSH used in the laboratory was from fasting blood samples, it would be better to evaluate the TSH level in fasting blood obtained in the morning than in random or postprandial samples.

Abbreviations

TSH, thyroid-stimulating hormone

Declarations

Ethics approval and consent to participate

The study had been performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Peking University First Hospital, and all the participants signed informed consent. The registration number of the study was ChiCTR2100047454 (18/06/2021).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable.

Authors' contributions

AMD conceived and coordinated the study, designed, performed and analyzed the experiments, wrote the paper. YYH, YCH and BJ carried out the data collection, data analysis, and revised the paper. All authors reviewed the results and approved the final version of the manuscript.

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Figures

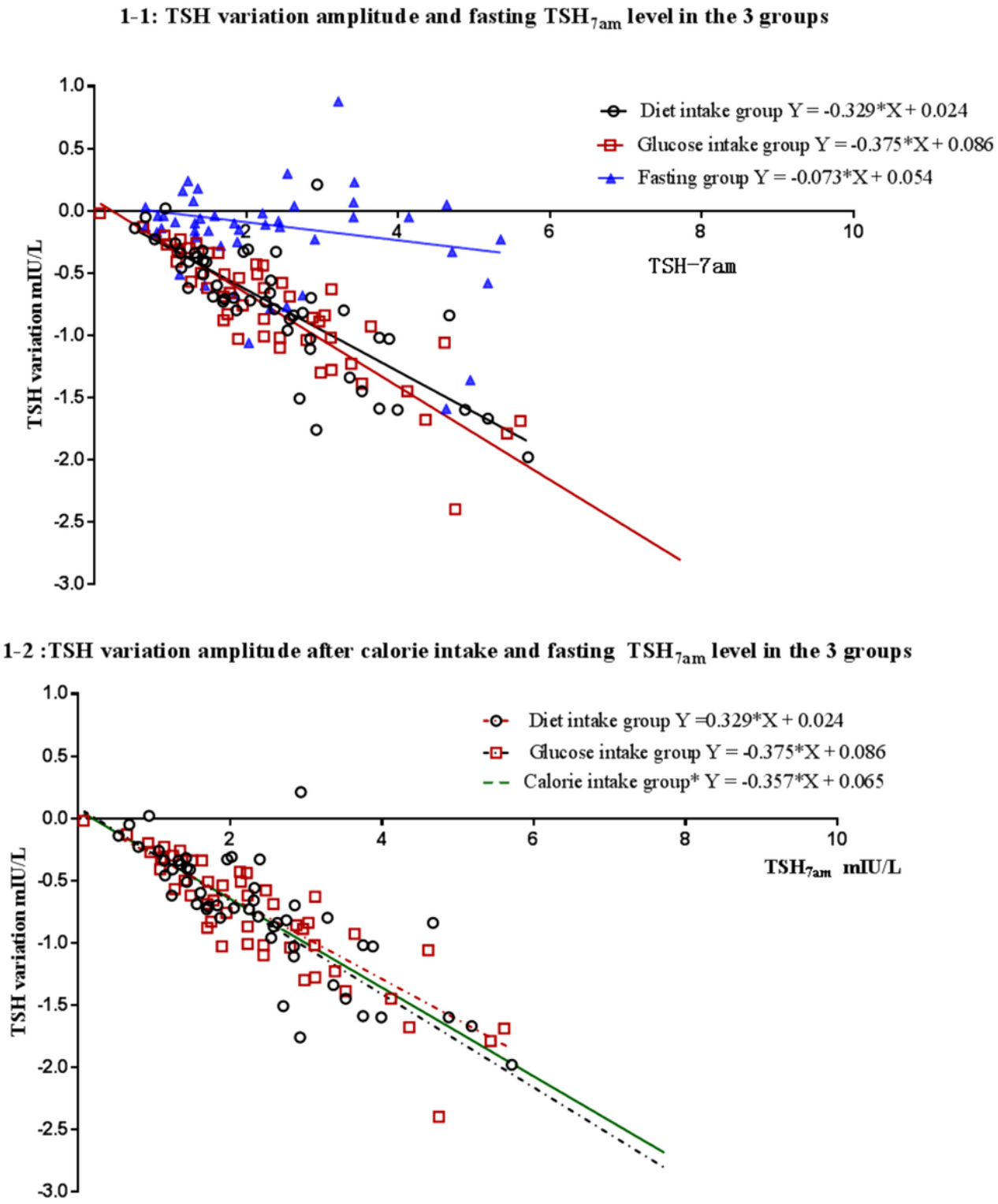

Figure 1

Correlation between TSH variation amplitude and fasting TSH7am level *Calorie intake group: data in the diet intake group and glucose intake group were combined together.

Supplementary Files

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