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The relationship between circulating lactate and islet hormones in non-obese women

Zależność między stężeniem mleczanu a stężeniem hormonów trzustkowych w osoczu u kobiet szczupłych i z nadwagą

Numerous studies have indicated that glucose metabolism in the adipose tissue contributing to 10-30% of whole body glucose metabolism provides a substantial amount of circulating lactate. In human adipocytes hyperinsulinemia and to a lesser extent hyperglycemia stimulate glucose conversion into lactate (3).

Elevated lactate production and release per fat cell has been demonstrated in overweight women and in first-degree relatives of individuals with type 2 diabetes in comparison with subjects without any heredity for diabetes (10). In obese and diabetic humans and animals adipose tissue can metabolize approximately 50-70% of taken up glucose into lactate and its production is positively correlated with fat cell size and the degree of obesity (6).

On the other hand, elevated blood lactate seems to affect the whole-body glucose metabolism. In rats lactate infusion has been found to induce insulin resistance in skeletal muscle by first suppressing the expression of genes involved in GLUT-4 synthesis, and thereafter inhibiting insulin-stimulated glucose uptake, altering the insulin signaling pathway and glycolysis (2).

The above data suggested that increased lactate production in adipocytes due to increased body fat seemed to affect a whole-body insulin action. However, according to our best knowledge data concerning the possible effect of fluctuating blood lactate due to different degree of body fat content on circulating glucagon are not available.

Hence this study was undertaken to examine the associations between plasma lactate concentrations and glucagon levels as well as between fasting lactate and circulating insulin to glucagon molecular ratio in non-obese females with different degree of body fat content.

MATERIAL

The participants were recruited on a voluntary basis in the local fitness club, however, their physical activity did not exceed 2 h a week. For participation 32 regularly menstruating females with BMI lower than 30 kg/m^2 were accepted. They were asked to follow their habitual diet at least 7 days prior to the participation and to refrain from physical activity for 48 h preceding blood collection. None of the subjects had health problems. They were non-smokers and did not take any medication on a regular basis. All the subjects gave their written consent prior to the participation. The experimental protocol was accepted by the Ethics Commission in the Academy of Physical Education. Three skinfolds

(triceps, subscapular, and supra-iliac) were measured using Harpenden caliper to calculate body fat (4).

ANALYTICAL METHODS

Blood was withdrawn after overnight fast at 8:00-8:30 a.m using disposable syringes and needles under aseptic conditions into EDTA tubes from subjects who had sat for at least 15 min. Plasma was separated by centrifugation (15 min/4000 rpm, 4^0 C) and stored at -70^0 until analyzed.

Plasma glucose was assayed by the oxidase method. Plasma lactate was assayed at 550 nm with the 4-aminoantipyrine and N-ethyl-N-(2hydroxy-3-sulphopropyl)-m-toluidine. Both variables were assayed using Randox commercial kits (Randox Laboratories,UK). Plasma insulin was determined by a standard radioimmunoassay technique with monoclonal antibodies against insulin using commercial kits (IRMA, OBRI-Orion, Poland). Intra- and inter- assay coefficients of variation (CV) were 6.8% and 9.3%, respectively. Glucagon was measured using the radioimmunoassay method and commercial kits (BioChem ImmunoSystem, Italy) with intra-and inter-assay coefficients of variation (CV) 8.8% and 8.7%, respectively. All measurements were done in duplicate.

Quantitive insulin sensitivity check index (QUICKI) was calculated using the formula QIUCKI = $1/[\log_{10} I_0 + \log_{10} G_0]$ where I_o and G_0 were fasting insulin and glucose levels, respectively (5).

STATISTICAL ANALYSIS

Data are presented as means ± s.d. Pearson product-moment correlations between plasma lactate levels and body fat, glucose levels, circulating insulin and glucagon as well as between plasma lactate and insulin to glucagon molecular ratio (I/G) were calculated. Due to non-Gaussian distribution I/G was logarithmically transformed ($\log_{10}$) before calculation. The limit of significance was set at $P<0.05$. Statistica v. 6.0 (StatSoft, USA) was used for all calculations.

RESULTS

Anthropometric characteristics of the subjects and fasting levels of biochemical variables are given in Table 1. Plasma glucose, insulin and glucagon concentrations in our participants were within physiological limits.

Plasma lactate concentrations were positively and significantly correlated with the percentage of body fat (Table 2). The inverse correlation between plasma lactate and insulin concentrations was close to the accepted limit of significance ($P<0.054$). The correlation between plasma glucagon and lactate concentrations, although non-significant, was slightly below the accepted limit of significance ($P<0.074$). However, significant and inverse relationship was noted between plasma lactate and I/G molecular ratio in plasma ($P<0.001$).

Table 1 Anthropometric and biochemical characteristics of the subjects

	Mean ± s.d.
Age (years)	30.7 ±12.4
Body mass (kg)	65.3 ±7.5
Body height (cm)	168.9 ±7.6
BMI (kg/m2)	22.9 ± 2.5
Fat (kg)	18.5 ±5.2
Fat (%)	28.4 ± 6.2
Insulin (pmol/L)	40.1 ± 16.4
Glucagon (pmol/L)	26.5 ±8.9
I/G*	1.7 ±0.9
QUICKI**	0.372 ± 0.032
Glucose (mmol/L)	4.5 ±0.4
Lactate (mmol/L)	2.1 ±0.6

Table 2 Pearson-product moment correlations between plasma lactate, percent of body fat, glucose and circulating pancreatic hormones in non-obese females

Variable	r	p
Fat (%)	0.460	0.008
Insulin (pmol/L)	- 0.340	0.054
Glucagon (pmol/L)	0.320	0.074
Log 10 I/G*	- 0.550	0.001
Glucose (mmol/L)	- 0.030	NS

*I/G - insulin to glucagon molecular ratio
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** - QUICKI- quantitive insulin sensitivity check index

DISCUSSION

In non-obese participants of the present study plasma lactate concentrations increased with body fat stores and this finding is in agreement with others' data (6).

The results of the present study indicated that in non-obese females circulating I/G molecular ratio is significantly and inversely correlated with in plasma lactate concentrations due to slight increase in circulating glucagon and decrease in circulating insulin levels.

It is well known that circulating insulin and glucagon undergo precise regulation by numerous factors including hormone secretion from the islet, its hepatic clearance and peripheral tissue sensitivity to insulin action (9). Additionally, it is worth noting that in dogs acute acidosis has been found to enhance plasma glucagon levels but to depress plasma insulin concentrations (1). On the other hand, there is a wealth of studies indicating that at least in vitro pancreatic β-cell function is sensitive to the changes in cytosolic pH and lactate/monocarboxylate transporters are expressed in pancreatic islets (8).

On the basis of our results the mechanism of lactate action on I/G ratio remains elusive. Taking into account that QUICKI values of our subjects were similar to that noted in subjects with normal glucose tolerance and insulin sensitivity it seemed feasible that peripheral insulin action was not disturbed in the participants of the current study (7). However, it could not be excluded that either endocrine islet function and/or hepatic clearance of pancreatic hormones is very sensitive to lactate action. In conclusion, is seems feasible that even a slight elevation in body fat and subsequent increased plasma lactate levels bring about alteration in circulating I/G ratio with metabolic consequences unknown at present.

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REFERENCES

1. Adrogue HJ, et al. Acidosis-induced glucose intolerance in not prevented by adrenergic blockade. Am J Physiol 1988; 255: E812-E823.
2. Choi ChS, et al. Lactate induces insulin resistance in skeletal muscle by supressing glycolysis and impairing insulin signaling. Am J Physiol 2002; 283: E233-E240.
3. Henry S, et al. Effects of hyperinsulinemia and hyperglycemia on lactate release and local blood flow in subcutaneous adipose tissue of healthy humans. J Clin Endocrinol Metab 1996; 81: 2891-2895.
4. Huygens W, et al. Body composition estimations by BIA versus anthropometric equations in body builders and other athelets. J Sports Med Phys Fitness 2002; 42: 45–55.
5. Katz A, et al. Quantitive insulin sensitivity check index: a simple accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab 2000;85; 2402-2410.
6. Konrad T,et al. Alpha-lipoic acid treatment decreases serum lactate and pyruvate concentrations and improves glucose effectiveness in lean and obese patients with type 2 diabetes. Diabetes Care 1999; 22: 280-287.

7. Labasa-Lhoret R, et al. Modified quantitive insulin check index is better correlated to hyperinsulinemic glucose clamp than other fasting-based index of insulin sensitivity in different insulin-resistant states. J Clin Endocrinol Metab 2003; 88: 4917-4923.
8. Lynch A, Best L. Cytosolic pH and pancreatic β-cell function. Biochem Pharmacol 1990; 40:411-416.
9. Moore MC, Cherrington AD. Regulation of net hepatic glucose uptale: interaction of neural and pancreatic mechanisms. Reprod Nutr Dev 1996; 36: 399-406.
10. Sandqvist MM, et al. Increased lactate release per fat cell in normoglycemic first-degree relatives of individuals with type 2 diabetes. Diabetes 2001; 50: 2344-2348.

ABSTRACT

In obese and diabetic humans and animals adipose tissue can metabolize glucose into lactate and its production is positively correlated with fat cell size and the degree of obesity. In rats lactate infusion has been found to induce insulin resistance in skeletal muscle by first suppressing the expression of genes involved in GLUT-4 synthesis, and thereafter inhibiting insulin-stimulated glucose uptake, altering the insulin signaling pathway and glycolysis. There is no in vivo studies concerning the association between plasma lactate and circulating glucagon levels. This study aimed at evaluation of the relationship between plasma lactate levels and circulating insulin and glucagons in non-obese female subjects. A total of 32 regularly menstruating females with BMI not exceeding 30 kg/m^2 volunteered to participate in the study. In all the subjects plasma insulin, glucagon and glucose were within respective physiological limits. Plasma lactate concentrations were positively and significantly correlated with the percent of body fat ($r=0.460$, $p<0.009$). Additionally, there were slight effects of plasma lactate on insulin and glucagon levels ($r=-0.360$, $p<0.054$ and $r=0.320$, $p<0.074$ for insulin and glucagon, respectively). In contrast, a significant and inverse correlation was noted between plasma lactate and circulating insulin to glucagon molecular ratio ($r=-0.550$, $p<0.001$). It is suggested that in non-obese women circulating insulin to glucagon molecular ratio is sensitive to the fluctuation in plasma lactate concentrations.

STRESZCZENIE

U ludzi i zwierząt z otyłością i cukrzycą typu II tkanka tłuszczowa ma zdolność przekształcania glukozy w mleczan, którego produkcja jest dodatnio skorelowana z wielkością komórek tłuszczowych oraz stopniem otyłości. U szczurów wykazano, że dożylne podanie mleczanu wywołuje oporność mięśni szkieletowych na insulinę, poprzez hamowanie ekspresji genów odpowiedzialnych za syntezę białka transportującego glukozę - GLUT-4 i stymulowanego przez insulinę transportu glukozy, ale także poprzez wpływ na molekularny mechanizm działania insuliny i przebieg glikolizy. Jednocześnie w dostępnym piśmiennictwie nie znaleziono danych dotyczących zależności między stężeniem mleczanu we krwi, a stężeniem glukagonu w warunkach in vivo. W niniejszych badaniach oceniano zależność między stężeniem mleczanu, a stężeniami insuliny i glukagonu w osoczu u regularnie miesiączkujących kobiet, których BMI nie przekraczało 30 czyli szczupłych i/lub z nadwagą. U wszystkich badanych stężenie inuliny, glukagonu oraz glukozy mieściło się w graniach norm fizjologicznych. Stężenie mleczanu we krwi było dodatnio i istotnie skorelowane z procentową zawartością tkanki tłuszczowej w całkowitej masie ciała ($r=0.460$, $p<0.009$). Ponadto zaobserwowano słabą korelację między stężeniem mleczanu a stężeniami insuliny i glukagonu (odpowiednio $r=-0.360$, $p<0.054$ i $r=0.320$, $p<0.074$). Jednocześnie wykazano ujemną i istotną korelację między stężeniem mleczanu, a stosunkiem stężenia insuliny do stężenia glukagonu stosunkiem osoczu ($r=-0.550$, $p<0.001$). Niniejsze badanie sugerują, że u kobiet szczupłych i z nadwagą stosunek stężenia insuliny do glukagonu w osoczu może ulegać wahaniom w zależności od stężenia mleczanu.