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High lactation index is associated with insulin sensitivity☆

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Abstract

The aim of the study was to evaluate the contribution of lactation to insulin sensitivity in women 12 to 18 month postpartum using an oral glucose tolerance test (OGTT). Mean lactation index (LI), a scoring system that considers the establishment and maintenance of the lactation was used. Lactation index was calculated according to the number of months of breast-feeding per child with a maximum of 72 points. The mean LI was calculated by dividing the total number of points by the number of children. A cutoff point of 72 was considered for the LI. We investigated the inverse of the homeostasis model assessment ($HOMA_{Sens}$) and the Cederholm index. Healthy women went through standardized interview and anthropometry. After a 10- to 12-h overnight fast, a 2-h OGTT was performed. Multiple regression analysis was performed with $HOMA_{Sens}$ and Cederholm index, which were adjusted for parity, percentage body fat, LI and presence/absence of breast-feeding. Both $HOMA_{Sens}$ and Cederholm index were negatively associated with percentage body fat ($P<.01$), and Cederholm index was positively associated with LI ($P=.01$). Mean 120-min insulin levels were significantly lower in women with LI=72 when compared with LI<72 women. Insulin sensitivity measured by the Cederholm index is positively associated with prolonged and sustained lactation, while percentage body fat presented a negative association. In this way, sustained lactation-associated metabolic changes are considered protective to women's health.

Keywords: HOMA; Cederholm index; Breast-feeding; Body adiposity

1. Introduction

Insulin sensitivity is an important parameter describing whole body glucose metabolism. The gold standard method to evaluate peripheral insulin sensitivity is the euglycemic hyperinsulinemic clamp [1], which is invasive and requires clinical facilities making it unsuitable for epidemiological studies. For this reason, there have been proposals for indices of insulin sensitivity derived from only fasting blood samples or from an oral glucose tolerance test (OGTT) [2–4]. Among the various methods to evaluate insulin sensitivity from fasting measurements, homeostasis model assessment (HOMA) [5] is the most commonly used, while among the indices proposed using postabsorptive measurements, the Cederholm index is based upon a physiological model and estimates the mean enhancement of glucose effectiveness due to plasma insulin during the test, correcting for the total body glucose space [6]. Generally, HOMA is commonly taken to represent central or hepatic insulin sensitivity, and the Cederholm index is indicative of peripheral insulin sensitivity.

Lactation has been previously shown to act as a protective factor to glucose metabolism in women, independent of body adiposity [7]. This is important because obesity is a major factor affecting insulin sensitivity [3,8]. Furthermore, lactation can reduce the risk of type 2 diabetes. In fact, it was shown [9] that the duration of breast-feeding was positively associated with a reduced incidence of type 2 diabetes in evaluation of two distinct cohorts of US nurses. In order to obtain a better understanding of the contribution of lactation to insulin sensitivity, we reexamined results of an OGTT performed in women 12 to 18 months postpartum [7]. We investigated the performance of $HOMA_{Sens}$ and the Cederholm index and propose an update to the calculation of the Cederholm index. To this end, we hypothesized that Cederholm index is capable to identify improvements in insulin sensitivity in women who have experienced sustained lactation. The objective of the study was to evaluate the contribution of lactation to insulin sensitivity.

2. Materials and methods

2.1. Subjects and protocol

The data used in the present study is part of a previously published study from our group [7] to which we refer for further details. In brief, healthy women were recruited to assessment by anthropometry and interview and to participate in an OGTT, according to standardized protocol. Women were recruited ($n=67$) from an outpatient

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Table 1
Socioeconomic, reproductive and health history from women according to LI[a]

	LI <72 (n=33; 49%)		LI=72 (n=34; 51%)		df	P[b]
	n	%	n	%		
SES						
High	7	21	10	29	1	.44
Low	26	79	24	71		
Gestational diabetes						
Yes	0	0	3	9	1	.08
No	33	100	31	91		
Parity						
Primiparous	8	24	22	65	1	.001
Multiparous	25	76	12	35		
Smoking						
Nonsmoker	28	85	31	91	1	.42
Smoker	5	15	3	9		
Still breast-feeding						
Yes	11	33	29	85	1	<.001
No	22	67	5	15		
Taking contraceptives						
Yes	18	55	11	32	1	.07
No	15	45	23	68		

SES, socioeconomic status.
[a] For details of subjects, selection and protocol refer to method section and reference [6].
[b] Statistical significance of effect (χ^2 test).

pediatric clinic at the University of Brasilia Hospital when bringing their infant for routine examination. Inclusion criteria were as follows: between 12 and 18 months postpartum, between 18 and 42 years of age and possessed one or more complete gestational cycles. Women were not recruited if any of the following exclusion criteria were present: twin and/or preterm (<38 weeks) birth; first complete gestational cycle under 18 years of age; use of drugs that could interfere with glucose metabolism (except oral contraceptives); health problems such as diabetes mellitus, hypertension, cancer, endocrine dysfunction or polycystic ovary syndrome. They were interviewed for detailed information about their health status and habitual life style. Anthropometry, according to standardized procedure, was conducted in the laboratory by the same investigator in the morning prior to the blood collection. Percentage of body fat was assessed with skinfold thickness using Siri's equation [10]. The OGTT was performed in the follicular phase of the menstrual cycle, after a 10- to 12-h overnight fast. An antecubital vein was cannulated and a blood sample collected at basal time (0 min). The cannula was kept patent with heparin (Liquemine; Produtos Roche Quimicos e Farmaceuticos, Rio de Janeiro, Brazil), in 1:10 dilution in NaCl solution (9 g/L). A 100-ml solution of 75% D-glucose (Dextrosol; Refinacoes de milho Ltda, Pouso Alegre, MG, Brazil) was ingested in 5 to 10 min. Then, the cup was twice filled with approximate 30 ml of water, which the subject consumed to ensure complete ingestion of the glucose dose. Blood samples were collected in appropriate evacuated tubes (Vacutainer; Becton Dickinson and Company, Franklin Lakes, NJ, USA) for serum and plasma (heparinized tubes) every 30 min for 2 h. Serum and plasma were harvested and stored at −18°C until analysis.

Plasma glucose (0, 30, 60, 90 and 120 min) was determined using a colorimetric enzymatic assay (Labtest Diagnostica, Belo Horizonte, Brazil). Serum insulin concentrations at the same time points were determined by radioimunoassay (DPC — Diagnostic Products Corporation, Los Angeles, CA, USA).

2.2. Calculations

Lactation index (LI) is a scoring system [11] devised to account for the establishment and maintenance of the physiological process of lactation over time. Each women was questioned about the length of time in months they breast-fed each infant and a score computed by adding points assigned to the number of months of breast-feeding per child: <1 month, 0 point; 1 to 5 months, 2 points/month; 6 months, 3 points/month; 7 to 9 months, 4 points/month; 9 to 11 months, 5 points/month; and ≥12 months, 6 points/month. Each mother could therefore obtain a maximum of 72 points for each child. The mean LI was calculated by dividing the total number of points by the number of children.

Glucose and insulin peaks (GP and IP, respectively) were defined as the highest concentration during the OGTT. Insulin sensitivity was calculated by the Cederholm index [5], with appropriate corrections to for the analytical matrix and serum insulin units. In the original work, serum glucose concentration is used in the numerator, but plasma glucose values are used in the denominator. For this work, unified units were employed, chosen to be the glucose plasma concentration (mmol/L) and insulin concentration (pmol/L). The expression for the Cederholm index was written as

$$CI = \frac{\left[\frac{D}{V_G} + [G(0)] - [G(T)]\right]}{\int_0^T [G(t)]dt \times log\left(\frac{1}{T}\int_0^T [I(t)]dt\right)}$$

In this expression, D is defined as the dose given (mmol), V_G represents the distribution space of glucose, taken to be 0.19 L/kg body weight, $G(t)$ and $I(t)$ are the plasma glucose and insulin concentrations measured between time 0 and time T. The unit

Table 2
Anthropometric and parameters of the OGTT from women according to LI

	LI <72 (n=33)		LI=72 (n=34)	
	Mean	84% CI[a]	Mean	84% CI
Actual age (y)	27.4	26.0–28.8	28.7	27.4–30.1
Body mass (kg)	59.8	57.0–62.6	56.5	54.2–58.9
Height (m)	1.57	1.55–1.58	1.57	1.56–1.59
Actual body mass index (kg/m^2)	24.2	23.2–25.1	22.8	21.9–23.7
Body fat (%)	29.4	28.0–30.9	28.1	26.7–29.5
Waist circumference (cm)	77.7	75.4–79.9	76.1	74.0–78.2
Waist/hip ratio	0.79	0.78–0.81	0.80	0.79–0.82
Basal glycemia (mmol/L)	4.25	4.11–4.38	4.64	4.53–4.75
120′ glycemia (mmol/L)	5.88	5.49–6.26	5.28	4.96–5.61
Peak glycemia (mmol/L)	7.67	7.32–8.02	7.34	7.04–7.65
Basal insulin (pmol/L)	51.8	39.0–64.6	34.7	28.8–40.6
120′ insulin (pmol/L)	392	282–502	210	176–244
Peak insulin (pmol/L)	588	508–668	487	401–572
Area glucose	782	732–832	728	703–753
Area insulin	49 863	39 352–60 373	36 930	31 104–42 757
$HOMA_{Sens}$	1.74	1.51–1.97	2.25	1.88–2.63
Cederholm index (min^{-1})	0.019	0.017–0.020	0.024	0.022–0.025

[a] CI, confidence interval.

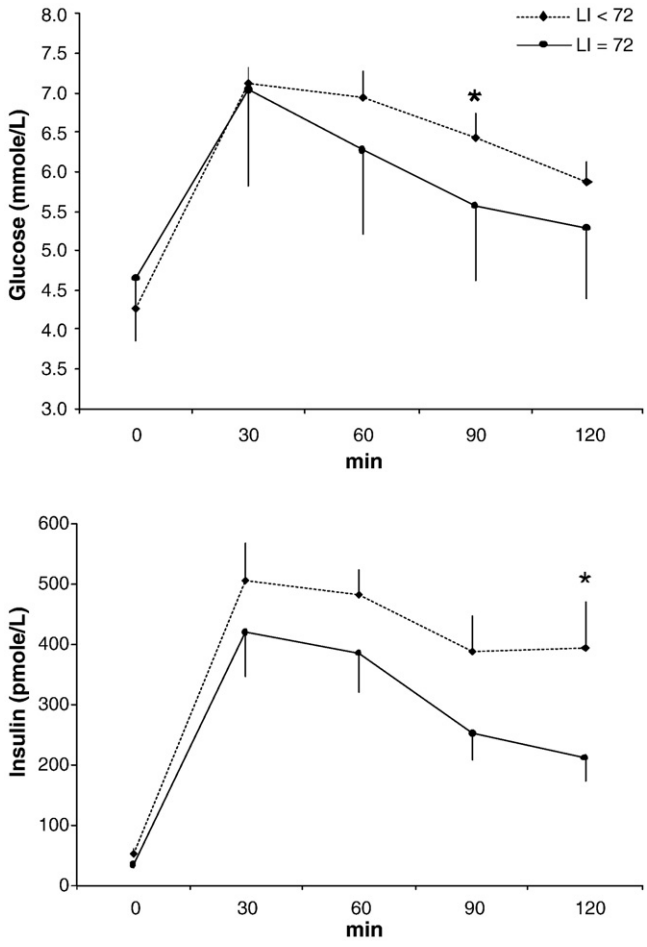

Fig. 1. Plasma glucose and insulin concentrations during OGTT in women according to LI (LI). Data are means plus or minus S.E. *Significantly difference by Mann–Whitney test between the groups (P<.05).

Table 3
Multiple regression analysis for log-transformed HOMA and Cederholm index of insulin sensitivity in women after 12 to 18 months postpartum

Model	Coefficient[a]	Confidence interval (95%)[a]		P
Cederholm index[b]				
Parity	0.86	−1.58	3.3	.48
LI	0.079	0.018	0.14	.01
Body fat (%)	−1.005	−1.22	−0.79	>.001
Still breast-feeding	−1.74	−4.81	1.33	.26
log $HOMA_{Sens}$[c]				
Parity	100.6	−238.2	439.4	.55
LI	0.75	−7.7	9.2	.87
Fat (%)	−40.6	−70.1	−11.2	.008
Breast-feeding	268.2	−158.4	694.8	.21

LI, Lactation Index.
[a] Coefficient and confidence interval values were multiplied by 1000 to improve table clarification.
[b] $F<0.0001$; $R^2=.61$.
[c] $F=0.048$; $R^2=.14$.

of this modified Cederholm index is min^{-1}. Integration was achieved using Simpson's rule to approximate the integral of the function using quadratic polynomials [12].

Insulin sensitivity was also measured by the inverse of the HOMA [5], which relies on computational modeling of basal glucose turnover rate for varying degrees of B-cell dysfunction and insulin resistance. The HOMA original formula was modified to accommodate the units for glucose (mM) and insulin (pM) as $156.26/I_0^* G_0$.

2.3. Statistical analysis

The χ^2 test was used to compare categorical variables. Continuous variables are presented as mean and 84% confidence intervals. Therefore, when the confidence intervals do not overlap, it means there is a statistically significant difference between the groups, which is an additional information as compared to a 95% confidence interval [13]. Variables presenting asymmetrical distribution, such as insulin parameters (basal, 120, peak and area), were logarithmically transformed prior to analysis.

A multiple regression analysis was performed using Intercooled STATA v. 9.1 (StataCorp LP, College Station , TX, USA) for Windows. Before performing the multiple regression analysis, distributions were tested for normality and a logarithmic transformation was applied where required. Multiple regression analysis was used to verify associations with the response variables, the Cederholm index and log ($HOMA_{Sens}$). The model was adjusted for parity, LI, percentage body fat and presence/absence of breast-feeding (explanatory variables). The reasons to include these explanatory variables in the model were their importance in explaining insulin sensitivity and presenting significant difference in the bivariate analysis according to LI. The level of significance used was 5%.

3. Results

The characteristics of the women enrolled in the study according to their LI are presented (Table 1). A cutoff point for the LI was set at 72 points, so to divide the group into high and low LI according to a long-term sustained lactation. There was no significant difference in the proportion of women in the two groups due to socioeconomic status, presence of gestational diabetes, smoking and use of oral contraceptives. There was a higher proportion of primiparous and women still breast-feeding in the group with LI of 72. Age and anthropometric parameters are not significantly different between the two groups (Table 2).

According to the criteria of the American Diabetes Association [14], none of the women were diabetic (2-h postload glucose >11.1 mM) but four women presented impaired glucose tolerance (IGT, 2-h postload glucose between 7.8 and 11.1 mM). There were three women with IGT (2-h glucose levels: 8.5, 8.8 and 10 mM) in the lower LI group and one woman (2-h glucose level: 7.9 mM) in the higher LI group. Removal of women presenting IGT did not change the full shape and characteristic of the groups' glucose and insulin response, so they were retained in the analysis (data not shown). The mean and confidence intervals for plasma glucose and insulin levels are presented in Table 2. Basal glycemia was higher in the group with high LI, and this group presented lower mean insulin values with the 120-min insulin level significantly lower when compared with women in the low LI group. Plasma glucose and insulin levels during the OGTT are presented in Fig. 1. Women with low LI presented significant higher glucose levels at 90 min and higher insulin levels at the end of the OGTT when compared to their high LI counterparts.

In the multiple regression analysis (Table 3), negative significant associations with the indices of insulin sensitivity, log($HOMA_{Sens}$) and Cederholm index are shown for percentage body fat. Lactation index was significantly and positively associated with Cederholm index. The regression model for the Cederholm index explained 61% of the associations, while the model with log($HOMA_{Sens}$) was less robust. The regression model also shows that the increase of fat mass contributes to a greater deal to explaining reduction in insulin sensitivity, and LI covers about 8% to the association in an opposite direction. That means that women who maintained a high LI are more insulin sensitive independent of their body fat.

4. Discussion

The negative association of percentage body fat with both indexes of insulin sensitivity examined in our model confirms that obesity is an important factor in the development of insulin resistance in young adult women. In our regression model, $HOMA_{Sens}$ was not significantly associated with LI. However, HOMA uses measurements taken in the basal state, and is therefore most indicative of the balance between hepatic glycogenesis and gluconeogenesis when fasting. Under the hyperinsulinemic and hyperglycemic conditions, characterizing the fed state glucose disposal into skeletal muscle becomes significant, and, unlike HOMA to which this has no contribution, [3] the Cederholm index strongly reflects peripheral uptake. On this basis, we believe that the comparison between the two measures indicates that the improvement in insulin sensitivity associated with prolonged breast-feeding is due to skeletal glucose utilization rather than hepatic glycogenic efficiency, i.e., glucose output.

An important aspect of our results is that lactation and adiposity are associated with insulin sensitivity in opposing directions. This indicates that the effect of lactation is not related to its influence on postpartum weight loss. In fact, a number of studies have pointed out that lactation alone does not appear to be sufficient to induce reduction in body adiposity [11,15,16]. Our model shows that even with the negative effect of increased body adiposity, lactation has a protective effect for a better response to an oral glucose load.

Factors known to positively affect improvements in insulin sensitivity are dietary changes and exercise, but these have to be dramatic to provide significant improvement in insulin sensitivity [17]. Therefore, only intensive lifestyle changes are capable to improve insulin sensitivity. Also, since the improvement in insulin sensitivity due to exercise rapidly disappears after its cessation, a continued program is needed [18]. The effect of lactation is continuous and prolonged and as shown in our model positive despite the presence of body adiposity. Additionally, Stuebe et al. [9] showed that an extended duration of breast-feeding per pregnancy was associated with greater benefit. They also suggest that the beneficial association begins to accrue after 6 months of lactation, supporting the idea that sustained lactation-associated metabolic changes have more profound effects on diabetes risk [9].

Levels of circulating insulin were lower in the group with prolonged and sustained lactation (LI=72) as compared to the group with lower LI. In fact lactating women are known to present lower insulin concentrations when compared to nonlactating controls [19], and reduced insulin levels in healthy subjects is taken as a marker for increased insulin sensitivity [20]. Studies in experimental animals have also shown improved insulin sensitivity, which is independent of weight change. Improved insulin sensitivity and glucose tolerance after three cycles of gestation and lactation have

been shown in rats in spite of increase in fat mass in the lactating group when compared to the group which had three cycles of gestation without lactation [21]. In our model, which controlled for the presence and absence of lactation, the improvement in insulin sensitivity is maintained if lactation is prolonged for up to 12 months, i.e., LI=72 points.

This work explores the association between lactation and improvement in insulin sensitivity in a cross-sectional design, which has limitations to prove causality. A randomized trial that would assign women to either short or long lactation periods is not feasible for ethical reasons. So additional evidence obtained from both cross-sectional studies in other regions and prospective cohorts of women is warranted to broadly characterize the effects of breast-feeding on glucose homeostasis.

In conclusion, insulin sensitivity measured by the Cederholm index is positively associated with prolonged and sustained lactation, while percentage body fat showed a negative association. This result can be viewed as another reassurance of the advantage of the lactation process to the metabolic adaptation and health protection of women.

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