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Subcutaneous adipose tissue measurements and better metabolic prediction

Research Article

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Abstract: The aim of the study was to establish the importance of an additional measurement of subcutaneous adipose tissue thickness (SAT) on a predetermined position on the waistline, and its relation to waist measurements as an improvement of metabolic prediction in equally obese subjects. One hundred and forty two consecutive patients were enrolled in the study: stratified by weight as normal (body mass index - BMI 20-25 kg/m^2), overweight (BMI 25-30 kg/m^2) and obese (BMI >30 kg/m^2); and by fasting glucose level as normoglycemic, impaired fasting glucose (IFG), or with type 2 diabetes mellitus (T2DM). SAT was measured in relaxed expiration, 3 cm left of the umbilicus, with ultrasound. Fasting blood samples for glucose, insulin and HbAlc were taken. Waist circumference was slightly higher in the IFG (112.8 cm) and normoglycemic groups (115.62 cm), compared to T2DM (108.15 cm). The T2DM group had a lower average SAT (2.7 cm) than both the IFG group (3.4 cm, $p<0.01$) and the normoglycemic group (4.2cm, $p=0.001$). The homeostatic model of assessment for insulin resistance (HOMA IR) was the lowest in normoglycemic and the highest in IFG group. Waistline radius to SAT ratio provides better insight into the deterioration of glucose metabolism than standard anthropometric markers of abdominal obesity in equally obese patients.

Keywords: *Subcutaneous adipose tissue thickness • Glucose metabolism deterioration • Central adiposity*

1. Introduction

Obesity is an emerging health problem closely associated with impaired glucose metabolism, arterial hypertension, accelerated atherosclerosis and neoplasia [1]. It is a chronic and progressive disease that requires lifelong intervention [2]. Its prevalence and many related chronic diseases make it an enormous economic burden [3].

In an article published in NEJM in 2007, Parnez *et al* suggested that there were 1.7 billion overweight and

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312 million obese people worldwide. In the previous 20 years the number of overweight and obese patients in westernized developing countries has tripled. Impaired glucose metabolism will will also become more prevalent. Indeed, it is predicted in the next 20 to 30 years that the number of people with IFG will rise from 197 to 420 million, and with T2DM from 171 to 366 million. Similarly, arterial hypertension is expected to increase in prevalence from 1 billion to 1.56 billion worldwide [4].

The main distinguishable types of obesity are abdominal (or android) and peripheral (gynoid). It is a well established fact that abdominal obesity is closely related to glucose metabolism impairment and cardiometabolic risk. Abdominal obesity comprises subcutaneous and visceral lipid compartments. The visceral compartment is defined as fat stores around viscera and intraperitoneally, with dorsal border of intestine and ventral surface of the kidney. Visceral lipid store accumulation is determined both by genetic predisposition and a positive energy balance [5].

Visceral lipid stores produce more free fatty acids after meals than subcutaneous adipose tissue. They also produce more polyunsaturated free fatty acids, whereas subcutaneous tissue produces more monounsaturated fatty acids [6]. Higher and altered postprandial free fatty acid fluxes from visceral lipid stores cause further metabolic impairment [7].

Adipocytes in central lipid stores produce less leptin and adiponectin than subcutaneous cells. Leptin has a role in satiety control and adiponectin is widely considered as an antiatherosclerotic agent. These cells synthesize more resistin that negatively influences insulin's action on target tissues. Alterations in adipokine synthesis in central adiposity causes obesity, insulin resistance, low grade inflammation and endothelial dysfunction [5].

Visceral lipid stores are related to elevated markers of inflammation [8]. Tumor necrosis factor alpha (TNFα) synthesis is elevated in visceral adipose tissue. It has a significant paracrine action that interferes with adipocyte metabolism and adipokine secretion. The inflammatory cytokine interleukin-6 (IL-6), which is also elevated, has ubiquitous proinflammatory effects and potentiates C-reactive protein (CRP) synthesis–a positive acute phase protein of the systemic inflammatory response that is associated with a higher cardiovascular risk. Higher levels of plasminogen activator inhibitor-1 (PAI-1) in people with central obesity lead to hypercoagulabilty and a higher cardiovascular risk [5].

All these events, combined with a sedentary lifestyle, have a detrimental effect on liver and muscle cell glucose metabolism. Lipotoxicity and low grade inflammation, together with low physical activity, result in a reduced action of insulin on target tissues (insulin resistance).

The development of insulin resistance through induced oversecretion by beta cells produces endoplasmatic reticulum stress and leads to a diminished insulin secretion and more beta cell apoptosis. Consequently glucose metabolism is impaired and the rising plasma glucose levels inhibit beta cell function and promote insulin resistance even further [9,10].

In essence, visceral lipid stores cause impaired glucose metabolism and enhance cardiovascular risk.

Some obese or overweight patients have a greater risk of metabolic disturbance than others, and this may be due to more centralized lipid stores [11]. In this context, we could add a new category – the benignly obese – who are less prone to obesity-related health consequences [12]. The key difference comes from the relationship between the mass of central and peripheral lipid stores.

As desirable as it seems, the precise measurement of centralized lipid stores is unfortunately not an easy task. Central lipid stores are usually determined using anthropometric measurements such as waist circumference, waist-to-hip or waist-to-height ratio. It is also possible to use bioelectric impedance (BIA), dual energy X-ray absorptiometry (DEXA), computed tomography (CT) or nuclear magnet resonance (NMR) [13]. The best option is to measure the areas of lipid stores after NMR imaging using custom software [14]. This is unfortunately far from manageable in everyday practice; therefore we need cheaper and easier methods to monitor centralization of lipid stores. From the previously mentioned anthropometric measurements, the best correlation to NMR results is seen with the waist-to-height ratio (WHtR) [15].

Obvious problems come from the fact that the waist circumference itself cannot predict the relationship between intraperitoneal and subcutaneous abdominal lipid stores, and we are not able to quantify delicate differences that may reveal those patients at higher risk caused by more centralized lipid stores. If we managed to use such information to improve early diagnosis and treatment of impaired glucose metabolism and endothelial dysfunction, it could prove useful.

It is possible to measure subcutaneous adipose tissue thickness with ultrasound and if we use a predetermined position on the waist line we will get a comparable result. Furthermore, if we combine the waist circumference with the measurement of subcutaneous adipose tissue thickness, their ratio would be a relative number that should reveal centralization of lipid stores and consequently the risk of metabolic disorder. The higher the result is, the more centralized the lipid store.

This result is comparable among different patients and for each particular patient over time.

The aim of this study was to establish whether the additional measurement of SAT on a predetermined position on the waistline, and its relation to the waist circumference, improves metabolic prediction in equally obese subjects.

Method: One hundred and forty two randomly selected overweight (BMI: 25-30 kg/m^2) and obese (BMI> 30kg/m^2) males admitted to the endocrinology outpatient clinic were enrolled in the study. The age range was 18 to 68. Subjects were stratified by fasting blood glucose level into normoglycemic, impaired fasting glucose (5.7 – 6.9 mmol/l) or diabetes mellitus type 2 (fasting glucose > 7.0 mmol/l or previously diagnosed).

Exclusion criteria were: age more than 70 years; hypothyroidism, including subclinical hypothyroidism (thyroid stimulating hormone > 5 μIU/mL); hypercorticism or using corticosteroid treatment; liver, heart or kidney failure; neoplasia; elevation of liver enzymes more than twice the upper limit; and diabetic patients taking insulin.

For every subject we measured height in cm, and weight in kg, using standard scales. Waist circumference was measured in cm in relaxed expiration using an elastic band around the imaginary line that passes over the umbilicus.

Subcutaneous adipose tissue thickness was determined in relaxed expiration 3 cm left of the umbilicus with ultrasound (Hewely Pacard Image point 9001) using a linear probe on 11 kHz (expressed in cm). Minimal pressure with the probe was applied during measurements.

Blood samples were taken after an overnight fast. Glucose was determined by the hexokinase method (ADVIA 1800 Siemens). Insulin levels were quantified using a chemiluminescent microparticle immunoassay (ARCHITECT i2000RS Abbot).

2. Calculation

Waistline radius and subcutaneous adipose tissue thickness ratio (WRSTR) was determined from the equation: WRSTR = waist circumference (cm) / 2π x SAT (cm).

Other equations used were:

HOMA IR = fasting blood insulin (IU/l) x fasting glucose (mmol/l) / 22.5. HOMA IS = fasting insulin (IU/L) x 20 / fasting glucose (mmol/l)–3.5.

Body mass index (BMI) = weight (kg) / height^2 (m^2)

3. Statistics

The results are presented as mean values ± standard deviation. Significant differences among the three study subject groups were analysed using one-way Analysis of Variance (ANOVA), with a *post hoc* Tukey test. Correlation between parameters was performed using Pearsons correlation. A *p* value of less than 0.05 was taken to be statistically significant. Data were processed using the software package Stat for Windows, R.4.5.

4. Results

Table 1 summarizes the values of all estimated parameters. There was a significant difference in the ages of the three groups. Patients with T2DM were older (mean 50.8 years) than those in the other groups (40.6 years in IFG group, 34.0 years in normoglycaemic group). There were also significant differences between the groups for weight (p< 0.01) and BMI (p<0.05). Data for waist circumference also showed slightly higher values for the normoglycemic (115.6 cm) and IFG groups (112.8

Table 1. Patients` demographic characteristics. Anthropometric and analytical data in the groups studied.

Parameters	Subject, mean value ± SD			ANOVA
	Normoglycemic	IFG	T2DM	
	N=34	N=49	N=59	
Age (yrs)	34.00 ± 11.03	40.60 ± 13.76	50.80 ± 11.24	p<0.001
Weight (kg)	107.40 ± 20.38	101.50 ± 20.76	94.00 ± 14.00	p<0.01
BMI (kg/m^2)	33.38 ± 6.69	31.99 ± 6.08	30.13 ± 4.04	p<0.05
Waist (cm)	115.62 ± 14.83	112.88 ± 13.91	108.15 ± 9.90	p<0.05
WHtR	0.64 ± 0.09	0.63 ± 0.08	0.61 ± 0.05	NS
SAT (cm)	4.21 ± 1.46	3.40 ± 1.28	2.71 ± 0.98	p<0.001
WRSTR	4.67 ± 1.29	5.88 ± 1.75	7.23 ± 2.65	p<0.001
HOMA IR	3.82 ± 2.78	5.69 ± 5.68	5.44 ± 4.03	NS
HOMA IS	198.30 ± 150.67	160.07 ± 167.67	68.48 ± 49.76	p<0.001

Table 2. Differences in SAT and WRSTR in normoglycemic, IFG and T2DM patients according to the BMI (patients with BMI 25-30 and BMI >30).

Parameters	Subject, mean value ± SD											
	Normoglycemic				IFG				T2DM			
BMI (kg/m^2)	25-30	>30			25-30	>30			25-30	>30		
No of patients	N=10	N=20	t	p	N=17	N=27	t	p	N=27	N=24	t	p
SAT (cm)	3.12 ± 0.77	4.95 ± 1.28	4.118	0.000	2.51 ± 0.77	4.00± 1.18	4.579	0.000	3.22 ± 1.00	2.39 ± 0.78	3.325	0.002
WRSTR	5.50 ± 1.12	4.17 ± 1.11	3.078	0.004	6.92 ± 1.64	4.96 ± 1.16	4.505	0.000	7.66 ± 2.60	6.17± 1.91	2.293	0.026

Table 3. Novel parameter correlations

	N	r	probability
Waist to WHTR	127	0.94	p< 0.001
WHTR to WRSTR	131	-0.42	p< 0.001
WRSTR to HOMA IS	130	-0.35	p< 0.001

cm) compared to T2DM (108.2 cm). SAT results showed significantly lower values for T2DM (2.7 cm) compared to IFG (3.4 cm, p<0.05) and normoglycemia (4.2 cm, p<0.001). The average WRSTR was 6.18. The WRSTR for T2DM was 7.23, higher than both the IFG group (p<0.01) and the normoglycemic group (5.88, p<0.001). On the edge of significance was the difference between the IFG and normoglycemic groups (p=0.059). The Average HOMA IR for all participants was 5.16, and there was no difference between the groups.

Differences in SAT and WRSTR in normoglycemic, IFG and T2DM patients according to the BMI are shown in the Table 2.

There was a significant positive correlation between the results for standard anthropometric markers of abdominal obesity waist and WHtR (p<0.001). A strong negative correlation between WHtR and WRSTR was also demonstrated (p<0.001). WRSTR also showed a strong negative correlation with HOMA IS (p<0.001). (Table 3).

5. Discussion

Age. There was a significant difference in age between the groups. Patients with T2DM were the oldest, the IFG group was younger and the normoglycemic group was the youngest. Bearing in mind the pathophysiological timeline of type 2 diabetes, this is an expected result in a randomly assembled group.

Weight. The group was predominantly obese with an average weight of 99.9 kg and BMI 31.6 kg/m^2. Although there was a significant difference among groups considering their weight (p< 0.05) and BMI (p<0.05), we should have in mind that our heaviest group were normoglycemic, so one may assume that the weight difference shouldn't alter metabolic results favorably in this group. Previously, weight and BMI were considered as the main parameters for obesity, but their predictive value for type 2 diabetes and cardiovascular disease seemed modest [16]. The lipid stores arrangement is more significant than just being obese or overweight [7]. Attention was shifted to abdominal obesity and consequently related central adiposity and its anthropometric markers waist circumference and waist-to-height ratio.

Waist and WHTR. Waist circumference is commonly used as a parameter for centralized obesity and it is found to be a good predictor of IR and metabolic syndrome [17]. According to the International Diabetes Federation, the cut-off for abdominal obesity in Caucasian men is 94 cm. This is more restrictive than an earlier cut-off point of 102 cm presented in the mid and late nineties [18,19]. However new insights in the correlation between waist circumference, metabolic syndrome and related cardiovascular diseases, together with the importance of timely intervention in obesity prevention and weight control justifies such change [20,21]. Data for waist circumference in our study showed slightly higher results in the IFG and normoglycemic groups compared to T2DM. Comparing only results for waist circumference, there is a significant difference (p<0.05) unfavorable to the normoglycemic group, but when adjusted by WHTR this difference disappears. This is an important result for this study because it shows that our groups were equally abdominally obese, at least using currently established anthropometrics. As previously mentioned, Wu *et al* found WHtR to be the marker with the best correlation to NMR measurements of central lipid stores [15]

Subcutaneous adipose tissue thickness. Although body weight and waistline measurements were similar, SAT results were significantly different between the groups (p< 0.05). This was however an expected result if we consider previous data and our clinical observations [22]. Having such differences in seemingly equally (at least abdominally) obese groups proved to us the value of an additional SAT measurement. Our goal was to look at SAT in relation to the waistline radius to achieve more informative and comparable results. We referred to this equation as WRSTR. Higher scores signify more centralized lipid stores.

WRSTR. For this equation we assumed the waist circumference to be circle, which of course it is not.

Nevertheless, this adaptation does not detract from its practical value. Using the circle circumference formula we were able to calculate subjects' waistline radius as waist circumference / 6.28 (2π). The WRSTR is the relationship between the approximate waistline radius and SAT, hence WRSRT = waist circumference / 2π x SAT. The average WRSTR in our study group was 6.18. For the T2DM group it was 7.23, for the IFG group 5.88 and for the normoglycemic group 4.67. This data showed a significant statistical difference between T2DM and IFG ($p<0.05$) and even stronger statistical difference between T2DM and normoglycemic groups ($p< 0.001$). On the edge of significance was the difference between the IFG and normoglycemic groups ($p=0.059$). Thus the additional measurement of SAT enabled better distinction among subjects of seemingly equal abdominal obesity, which was our primary goal. Putting the waistline and SAT in one equation seems logical since we are looking for a way to intergrate data gathered from both abdominal obesity andSAT thickness.

HOMA IR and HOMA IS. Average HOMA IR for all participants was 5.16. It was the lowest in the normoglycemic group (3.82) and highest in the IFG group (5.69). The better than expected result in T2DM (5.44) is probably secondary to medications. Since IFG and T2DM are practically the same pathophysiological condition, differing only in the magnitude of damage to the beta cell pool, it is not surprising that there is less difference between them than when we compare them to the normoglycemic group. The differences between the groups were not statistically significant. The importance of adipose tissue centralization for insulin resistance is potentiated by the lowest HOMA IR in the most obese – the normoglycemic group – which is the group that had the lowest centralization of lipid stores according to WRSTR. The fact that all groups are insulin resistant could probably be explained by the overall obesity in our study group. Gradually the beta cell pool weakens and dies out [23]. As expected, HOMA IS as a marker of beta cell pool power was highest in the normoglycemic group (198.30, *vs.* 160.1 in IFG, and 68.5 in T2DM, $p<0.05$ for both).

There was a significant correlation between the results for standard anthropometric markers of abdominal obesity, waist circumference and WHtR ($p<0.001$). We also found a strong negative correlation between WHtR and WRSTR ($p<0.001$). This suggests that WRSTR is influenced by the degree of obesity and that should be considered when results are compared. It seems that WRSTR is better designed for the follow-up of individual patients and the analysis of groups of subjects that don't differ markedly in waist circumference.

Probably the most interesting data for further investigation acquired in this study comes from the correlation between WRSTR and HOMA IS ($p<0.001$). Potentially, early detection of subjects prone to impaired glucose metabolism, possibly using WRSTR, as one of goals presented by the international group for pre-diabetes [24] could provide us with patients that still have enough beta cells to preserve. This is important since in humans there is no beta cell turnover after 30 years [25,26]. Today, beta cell rest with exogenous insulin is an emerging therapeutic strategy in treating beta cell dysfunction, particularly in early T2DM. Relief with exogenous insulin allows time for cellular reparatory mechanisms to counter the negative effects of prolonged endoplasmatic reticulum stress in the beta cell. Long-term remission of glucose metabolism deterioration, with restoration of beta cell function were reported. [27-30]. Early detection of patients prone to incipient beta cell failure by monitoring abdominal obesity centralization perhaps using WRSTR in normoglycemic, overweight and obese subjects, followed by intermittent and careful implementation of beta cell rest, might give us new hope in the ongoing battle against type 2 diabetes mellitus.

6. Conclusion

After analyzing previous data we can conclude that relatively simple and inexpensive additional procedures provide significantly better information in seemingly equally obese or overweight subjects. We were able to distinguish metabolic differences between patients where strandard antropometric measurements could not provide such information. Additional benefit comes from the possibility to monitor the dynamics of lipid store centralization over time and hopefully predict metabolic deterioration before substantial damage occurs. This will be the focus of further research.

List of abbreviations

SAT – subcutaneous adipose tissue.
BMI – body mass index.
IFG – impaired fasting glucose.
T2DM – diabetes mellitus type 2.
HOMA IR – homeostatic model of assessment for insulin resistance.
HOMA IS – homeostatic model of assessment for insulin secretion.
PAI-1 – plasminogen activator inhibitor-1.
WHtR – waist-to-height ratio.
WRSTR – waistline radius and subcutaneous adipose tissue thickness ratio.

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