PANCREATIC FAT IS ASSOCIATED WITH METABOLIC SYNDROME AND VISCERAL FAT BUT NOT BETA-CELL FUNCTION OR BODY MASS INDEX IN PEDIATRIC OBESITY

Johan Staaf, MD, ‡‡ Viktor Labmayr, MD, ‡ Katharina Paulmichl, MD, §§ Hannes Manell, MD, ‡‡
Jing Cen, MD, ‡ Iris Ciba, MD, ‡‡ Marie Dahlbom, RN, ‡‡ Kirsten Roomp, PhD, ¶¶ Christian-Heinz Anderwald, MD, MPH, MBA, ‡‡‡ Matthias Meisnützer, MD, ** Reinhard Schneider, PhD, ¶¶ Anders Forslund, MD, PhD, ‡‡ Kurt Widhalm, MD, §§ §§ Jonas Bergquist, MD, PhD, §§§ Håkan Ahlström, MD, PhD, §§§ Peter Bergsten, MD, PhD, * Daniel Weghuber, MD, §§§ and Joel Kullberg, PhD §§§

Objective: Adolescents with obesity have increased risk of type 2 diabetes and metabolic syndrome (MetS). Pancreatic fat has been related to these conditions; however, little is known about associations in pediatric obesity. The present study was designed to explore these associations further.

Methods: We examined 116 subjects, 90 with obesity. Anthropometry, obesity-related metabolic features of the cohort.

Results: The PFF was elevated in subjects with obesity. No association between PFF and body mass index-standard deviation score (BMI-SDS) was found in the obesity subcohort. Pancreatic fat fraction correlated to Insulin Secretion Sensitivity Index-2 and Homeostatic Model Assessment of Insulin Resistance in simple regression; however, when using adjusted regression and correcting for BMI-SDS and other fat compartments, PFF correlated only to visceral adipose tissue and fasting glucose. Highest levels of PFF were found in subjects with obesity and MetS.

Conclusions: In adolescents with obesity, PFF is elevated and associated to MetS, fasting glucose, and visceral adipose tissue but not to beta-cell function, glucose tolerance, or BMI-SDS. This study demonstrates that conclusions regarding PFF and its associations depend on the body mass features of the cohort.

Key Words: pancreatic fat, pediatric obesity, beta-cell function, metabolic syndrome, body mass index-standard deviation score

Pancreas 2017;46: 358–365

The prevalence of pediatric obesity and type 2 diabetes mellitus has increased in recent decades.1,2 Obesity-related metabolic complications are associated with distribution and amount of lipids stored in adipose tissue compartments.3 Accumulation of lipids in subcutaneous adipose tissue (SAT) is complemented with storage in visceral adipose tissue (VAT) and other ectopic sites such as the liver and pancreas.3 Pancreatic fat fraction (PFF) can be measured by magnetic resonance imaging (MRI) and increase with body mass index (BMI) both in adults3 and adolescents.4 In addition, elevated PFF has been associated with age, male sex, and Hispanic ethnicity.7,8 Pancreatic fat is located in close proximity to insulin-secreting beta-cells and is a part of the total ectopic fat deposition, which implies that it could potentially be associated to both beta-cell function (BCF) and insulin resistance (IR). Indeed, pancreatic fat has been connected to impaired BCF in both adults9,10 and youth.10 In contrast, other previous studies have found no such relationship.5,8,11 Furthermore, several studies report associations between high PFF and IR as well as impaired glucose metabolism.6,9,10,12 A gradual increase in PFF between subjects with normal glucose tolerance (NGT), impaired fasting glucose (IFG), and impaired glucose tolerance (IGT) has been demonstrated.13 The highest levels of PFF have been found in subjects with diabetes.9,14

The prevalence of MetS in adults has been estimated at approximately 20% to 30% and at 12% to 30% in adolescents.2,15,16 In addition, elevated levels of PFF have been linked to MetS in both adults12,17 and adolescents.5 Furthermore, disturbed levels of proinsulin as well as the proinsulin-to-insulin (P/I) ratio have been proposed as possible biomarkers of beta-cell dysfunction and impaired glucose regulation. These biomarkers have also been
associated to MetS\textsuperscript{18,19}; however, their relation to PFF has yet not been studied. This study had 4 main objectives. These were to carefully measure PFF in children and adolescents with and without obesity and to 1. relate it to indices of BCF and IR; 2. explore a previously reported correlation between PFF and MetS; 3. examine whether PFF differs between subjects with NGT and IFG/IGT; and 4. study the correlation between PFF and other body composition measurements including MRI-based quantification of VAT, SAT, and liver fat fraction (LFF) and BMI-standard deviation score (SDS). The results and discussion sections are structured to follow the order of these objectives.

MATERIALS AND METHODS

Study Population

This dual-center cross-sectional study was carried out at Uppsala University Hospital, Sweden and Paracelsus Medical University Hospital in Salzburg, Austria. Children and adolescents (N = 125) aged 10 to 18 years and part of the beta-JUDO (beta-cell function in Juvenile Diabetes and Obesity) cohort (FP7-HEALTH-2011-two-stage, project number: 279153) were included based on age. Subjects unable to comply with the MRI and oral glucose tolerance test (OGTT) protocols were excluded. Furthermore, 9 subjects were excluded because of missing PFF or OGTT datasets, leaving 116 subjects with complete datasets to be further analyzed. Ninety (44 female) subjects were characterized with obesity (BMI-SDS ≥ 2.0) and 26 (12 female) as nonobese (BMI-SDS < 2.0).\textsuperscript{20,21} The latter group is not denoted controls because it includes both overweight and underweight subjects. Examinations were harmonized between study centers. Written parental and oral consent from children as well as ethical approval from local ethics committees were obtained.

Anthropometric and Blood Pressure Measurements

Height and weight were assessed by standardized and calibrated scales (Seca, Hamburg, Germany) and stadiometers (Uppsala: Ulmer (Busse Design + Engineering GmbH; Elchingen, Germany); Salzburg: Seca). The BMI-SDS was calculated with Microsoft Excel add-in LMS Growth using WHO growth report.\textsuperscript{21} Waist circumference was measured midway between the superior border of the iliac crest and lowest rib. Systemic blood pressure was measured using a standardized clinical aneroid sphygmomanometer (Uppsala: CAS 740; CAS Medical Systems, Inc, Branford, Conn; Salzburg: Careseas V100; Dinamap Technology/GE, Vienna, Austria).

Blood Sampling and Analyses

Blood was sampled at fasting using an intravenous stationary catheter. Glucose, triacylglycercides (TG) and high-density lipoproteins (HDL) were analyzed according to local protocols. In Uppsala, glucose was analyzed using an Architect c8000 instrument (Abbott Diagnostics, Solna, Sweden) and by a Glucouquant Glucose-Kit (Roche Diagnostics, Mannheim, Germany) in Salzburg. Uppsala quantified TG and HDL using an Architect c800 instrument (Abbott Diagnostics) and in Salzburg an enzymatic photometric test (Modular Analytics System, P-Modul, 917; Roche Diagnostics) was used. Validation of analyses was performed between the laboratories in Uppsala and Salzburg using reference blood samples. Selected samples underwent immediate centrifugation at 2500g for 10 minutes at 4°C, subsequently aliquoted, and frozen at −80°C. Plasma was later used for central analyses of insulin, proinsulin, and C-peptide in Uppsala. Single-plex enzyme-linked immunosorbent assay kits for each analyte were used (Merodia AB, Uppsala, Sweden). Standardized control samples (Merodia AB) were used to control for interplate variability.

Oral Glucose Tolerance Test

The OGTTs were conducted as previously described.\textsuperscript{22} Subjects were instructed to drink a 300-mL glucose solution (1.75 g glucose per kilogram body weight; maximum 75 g glucose) within approximately 5 minutes. Subsequently, blood samples were obtained from a stationary venous catheter at predetermined time points after the glucose challenge.

Definition of Metabolic Syndrome and Prediabetes

The MetS was defined according to the International Diabetes Federation consensus for children and adolescents.\textsuperscript{23} Prediabetes was defined as IFG and/or IGT according to the American Diabetes Association\textsuperscript{24} because of the same fasting glucose cutoff level (25.6 mmol) as in MetS.

Beta-cell function and IR

To estimate BCF and IR, several indices were used. Homeostatic model assessment (HOMA)-beta and PI were derived from fasting measurements.\textsuperscript{25,26} First-phase insulin secretion during OGTT was evaluated by insulinogetic index (IGI) using 15 and 30 minutes as well as both insulin and C-peptide in the numerator.\textsuperscript{27} The area under the curve (AUC), calculated using the trapezoid rule, for glucose, insulin, and C-peptide was used to represent both total and dynamic secretory response for the entire OGTT period. Dynamic AUC was calculated as the AUC above baseline (t=0). The BCF corrected for insulin sensitivity was assessed by the oral disposition index (IGI × Matsuda index) and the insulin secretion sensitivity index-2 (ISSI-2) ([AUCinsulin/AUCglucose] × Matsuda index).\textsuperscript{10,28} The IR was assessed by HOMA-IR, 1/fasting insulin, and by the Matsuda index.\textsuperscript{26,28}

Magnetic Resonance Imaging

The MRI examinations were performed to determine PFF, LFF, and volumes of abdominal VAT and SAT. All examinations were performed using 1.5 Tesla clinical MRI systems from Philips Medical System (Best, The Netherlands). Uppsala used a Philips Achieva system, whereas Salzburg used a Philips Ingenia system. Uppsala served as core laboratory and developed and standardized the imaging protocol at both sites and performed all image analyses.

Imaging was conducted in a supine position after a standardized light meal in proximity to the OGTT, preferably on the same day. All MRI assessments used water-fat imaging techniques during breath hold. The pancreas scan was performed using surface coils, and the LFF and VAT/SAT scans were performed using the built-in body coils. The pancreas scan was tilted (−15 degrees around the coronal direction) for better pancreas coverage.

For both pancreas and liver, the water-fat image reconstruction was performed using a multiresolution version of a previously described method that uses a whole-image optimization approach.\textsuperscript{29} The reconstruction used the same TG spectrum model,\textsuperscript{30} a common R²* parameter, and a regularization parameter (μ = 10). The water and fat image reconstruction was performed using the last 5 echoes only because phase errors in the first echo were seen to significantly affect the results.\textsuperscript{31,32} The first and last slices in each volume were excluded from the analysis because the image contrast in these slices differed.

An experienced operator performed the measurements using manual segmentation. The segmentations were performed in the...
Pancreatic Fat Fraction and MetS

To investigate association between PFF and MetS, the study population was divided into 3 groups: subjects without obesity (group 1), subjects with obesity but without MetS (group 2), and subjects with obesity and MetS (group 3). The groups did not differ with regard to age or sex, and groups 2 and 3 did not differ with regard to BMI-SDS (data not shown). As depicted in Figure 1, a gradual increase in PFF was shown between groups. Group 1 (n = 26): 1.05 [−0.4–3.3]; group 2 (n = 66): 2.2 [−0.6–11.8]; and group 3 (n = 24): 3.65 [1.2–14.6%] (1 vs 2: P = 0.002; 2 vs 3: P = 0.016). In group 3, IR, fasting insulin, and proinsulin levels were also significantly elevated (data not shown). Systolic blood pressure and triglyceride levels were elevated in subjects with obesity, whereas no difference in HDL or fasting glucose levels was detected between subjects with and without obesity (Table 1). After adjustment for VAT, the association of PFF and MetS had a P value of 0.051 between the groups with obesity and 0.058 between all groups.

Pancreatic Fat Fraction and Glucose Metabolism

Fasting glucose was associated with PFF in subjects with obesity (Table 2). Subjects with IFG had higher PFF compared with subjects with NGT (3.4% vs 2.2%, P = 0.026). Moreover, a trend was observed between PFF and the 2-hour glucose level in obese children (Table 2). The PFF did, however, not differ between obese NGT and obese prediabetic subjects (IFG and IGT) (P = 0.105), and no difference was detected between obese IFG and IGT (P = 0.276). In subjects without obesity, a negative correlation between PFF and fasting glucose was observed (Table 2). When adjusted regression modeling was used, only PFF and fasting glucose in subjects with obesity remained significantly correlated (r = 0.243, P = 0.020).

Pancreatic Fat Fraction, Other Fat Compartments, and BMI-SDS

In the whole cohort, PFF was positively correlated to VAT, SAT, and liver fat (Table 2). However, VAT was the only significant parameter in adjusted models (model A: r = 0.289, P = 0.021; model B: r = 0.340, P = 0.027). In addition, when subjects with obesity were examined, only VAT showed correlation to PFF in simple regression models. When examining ectopic fat deposition in the pancreas and liver, a positive correlation was found in subjects without obesity. In subjects with obesity, no association was found (Table 2) (Fig. 2). The BMI-SDS correlated to amount of PFF in the whole cohort and in subjects without obesity; however, no correlation between BMI-SDS and PFF in subjects with obesity was found (Table 2) (Fig. 3).

Cohort-Dependent Results

Associations between PFF and other parameters were frequently found dependent on the cohort examined (Table 2). Comparing the whole cohort with the obesity subcohort, 12 associations to PFF were differently correlated. These differences were found
within all areas examined: BMI-SDS, BCF, IR, MetS components, glucose metabolism, and fat compartments (Table 2).

**DISCUSSION**

Fat accumulation in the pancreas could potentially have an effect on insulin-producing beta-cells, both directly through lipotoxicity mediated by the release of free fatty acids and indirectly through activation of inflammatory pathways. Indeed, PFF has been shown to correlate with pancreatic TG content and circulating free fatty acids. However, whether pancreatic adipocyte infiltration reflects intra-islet fat content and whether adipose tissue within the pancreas has a profound effect on insulin-secreting beta-cells remain unclear. When we, in this study, compared pediatric subjects with and without obesity, significant differences could be found in PFF as well as in parameters of BCF and IR (Table 1). However, when simple regression analyses were performed, only few parameters of BCF showed an association to PFF (Table 2). The ISSI-2 correlated to PFF in subjects with obesity, confirming previous results in youth. Cohen et al investigated BCF and IR in juveniles with PFF absent and PFF present, respectively. The PFF-present group had impaired BCF and elevated IR; however, only 29% of the subjects were found in this group, which must be considered when interpreting the results. Indeed, Cohen et al showed a relation between PFF and ISSI-2 in a group comparison, but when adjusting for BMI-SDS and VAT, no correlation between PFF and any indices of BCF remained significant in this study. This also agrees with recently published data in adults. These previous findings and our concurring data lead us to conclude that PFF is not directly associated to BCF in childhood.

Interestingly, PFF correlated to fasting proinsulin and AUCproinsulin in the whole cohort, indicating that elevated PFF could potentially contribute to beta-cell stress and disturbed intra-cellular insulin processing. Proinsulin has previously been related to beta-cell dysfunction. However, when analyzing subcohorts separately, no correlations remained indicating that the positive correlations may be due to group differences. In addition, in the whole cohort, PFF was related to IR verifying previous results of ectopic fat relating to IR. Nonetheless, in obese children,
only HOMA-IR remains significant, and in adjusted regression models, no significant correlations were found (Table 2). Therefore, we conclude that PFF is not a marker of proinsulin secretion or the result of IR in adolescence. Other depots such as visceral fat and hepatic fat are likely to be more strongly associated to IR.6

Obesity is associated with increased risk of MetS.34 In the present study, PFF was significantly higher in subjects with obesity and MetS, confirming previous studies both in adults12 and adolescents.5 However, Lee et al12 used computed tomography to quantify PFF in adults, whereas Maggio et al5 included only 5 adolescents with MetS. We found that both PFF and VAT were elevated in subjects with obesity and MetS and related to each other. The PFF was not independently associated to MetS when adjusting for VAT ($P = 0.051$), indicating that VAT constitutes a link. On the other hand, we acknowledge a very strong trend between PFF and MetS, independently of VAT, that challenges

|TABLE 2. Simple Spearman Rank Correlations Between Pancreatic fat fraction and Phenotype Data |
|---|---|---|---|
|Pancreatic fat fraction | Whole Study Group (n = 116) | Obese (n = 90) | Nonobese (n = 26) |
| | $r_{Spearman}$ | $P$ | $r_{Spearman}$ | $P$ | $r_{Spearman}$ | $P$ |
|Age | $-0.059$ | 0.526 | $-0.074$ | 0.486 | $-0.181$ | 0.377 |
|BMI-SDS | **0.276** | 0.003 | 0.021 | 0.841 | **0.399** | 0.043 |
|Beta-cell function | | | | | | |
|IGI 15 minOGTT insulin | 0.091 | 0.345 | 0.016 | 0.884 | $-0.118$ | 0.600 |
|IGI 30 minOGTT insulin | 0.106 | 0.266 | 0.017 | 0.875 | 0.013 | 0.951 |
|IGI 15 minOGTT c-peptide | 0.056 | 0.564 | $-0.023$ | 0.854 | $-0.076$ | 0.737 |
|IGI 30 minOGTT c-peptide | 0.038 | 0.691 | $-0.046$ | 0.670 | 0.087 | 0.672 |
|HOMA-beta | 0.183 | 0.053 | $-0.004$ | 0.969 | 0.177 | 0.397 |
|oD1 | $-0.126$ | 0.194 | $-0.133$ | 0.232 | 0.278 | 0.178 |
|ISSI-2 | **-0.242** | **0.012** | **-0.301** | **0.006** | 0.311 | 0.131 |
|AUC insulin | 0.177 | 0.057 | 0.074 | 0.490 | $-0.211$ | 0.302 |
|AUC proinsulin | **0.183** | **0.050** | 0.094 | 0.378 | $-0.278$ | 0.169 |
|AUC c-peptide | 0.170 | 0.068 | 0.063 | 0.555 | $-0.134$ | 0.516 |
|Dynamic AUC insulin | 0.159 | 0.088 | 0.050 | 0.639 | $-0.159$ | 0.439 |
|Dynamic AUC proinsulin | 0.157 | 0.093 | 0.076 | 0.475 | $-0.288$ | 0.153 |
|Dynamic AUC c-peptide | 0.098 | 0.296 | $-0.020$ | 0.852 | $-0.097$ | 0.639 |
|P/I fasting | $-0.022$ | 0.814 | $-0.062$ | 0.561 | $-0.035$ | 0.866 |
|P/I 15 minOGTT | 0.092 | 0.328 | 0.074 | 0.489 | $-0.173$ | 0.409 |
|P/I 30 minOGTT | $-0.052$ | 0.577 | $-0.056$ | 0.602 | $-0.227$ | 0.265 |
|Insulin sensitivity | | | | | | |
|HOMA-IR | **0.284** | **0.002** | **0.211** | **0.047** | $-0.219$ | 0.282 |
|Matsuda index | $-0.271$ | 0.005 | $-0.210$ | 0.057 | 0.260 | 0.209 |
|1/Insulin | **-0.268** | **0.004** | $-0.179$ | 0.091 | 0.116 | 0.574 |
|MetS components | | | | | | |
|Waist circumference | **0.312** | **0.001** | 0.108 | 0.327 | 0.308 | 0.127 |
|Systolic blood pressure | **0.210** | **0.026** | 0.037 | 0.734 | 0.279 | 0.168 |
|Diastolic blood pressure | 0.124 | 0.191 | 0.150 | 0.168 | 0.008 | 0.968 |
|Triglycerides | **0.193** | **0.038** | 0.019 | 0.860 | 0.098 | 0.632 |
|HDL | $-0.122$ | 0.196 | $-0.173$ | 0.105 | 0.209 | 0.316 |
|Fasting glucose | 0.107 | 0.257 | **0.290** | **0.006** | $-0.527$ | **0.006** |
|Glucose metabolism | | | | | | |
|Glucose 120 min | 0.155 | 0.099 | 0.166 | 0.120 | $-0.088$ | 0.675 |
|Fasting insulin | **0.268** | **0.004** | 0.179 | 0.092 | $-0.114$ | 0.578 |
|Fasting proinsulin | **0.237** | **0.011** | 0.131 | 0.218 | $-0.202$ | 0.324 |
|Fasting C-peptide | **0.325** | <**0.001** | **0.223** | **0.035** | 0.100 | 0.627 |
|Fat compartments | | | | | | |
|VAT | **0.414** | <**0.001** | **0.223** | **0.034** | **0.472** | **0.015** |
|SAT | 0.272 | **0.003** | 0.041 | 0.701 | 0.294 | 0.145 |
|Liver fat | 0.273 | **0.003** | 0.033 | 0.758 | **0.480** | **0.013** |

Bold letters indicate significant univariate correlations.

*Significant results after controlling for significant covariates using models A and B, respectively. Model A includes age and BMI-SDS and model B includes VAT, SAT, and liver fat.
previous findings\textsuperscript{5} and warrants further investigations. Generally, subjects with MetS have an increased risk of type 2 diabetes mellitus.\textsuperscript{3,4} In adults, PFF has been shown to be highest in subjects with diabetes.\textsuperscript{14} Recent pediatric studies have demonstrated that prediabetic subjects have higher PFF.\textsuperscript{5,35} However, when adjusted regression analyses were performed by Pacifico et al.\textsuperscript{6} only liver fat remained significantly correlated to prediabetes. In the present study, no significant relationship between PFF and IGT was found; however, we demonstrate a positive correlation between PFF and fasting plasma glucose as well as PFF and IFG, which need further longitudinal investigation in larger cohorts with regard to causality and pathophysiological impact.

We found that LFF was approximately 4 times higher and PFF at least 2 times higher in subjects with obesity (Table 1). Previous studies have reported a weak or nonexistent correlation between ectopic fat deposition in the pancreas and liver.\textsuperscript{5,9} Here, we observe a correlation between these fat compartments in subjects without obesity, suggesting that early deposition of ectopic fat is not organ specific. Indeed, PFF has also previously been associated to hepatic steatosis in adults.\textsuperscript{36} We also discovered a positive correlation in the whole cohort, but interestingly not within the obesity subcohort (Fig. 2), implying that once obesity is overt, ectopic fat deposition is more organ selective, possibly because of genetic factors.\textsuperscript{37} Our results are in agreement with data reported by Pacifico et al.\textsuperscript{6} Furthermore, we report that PFF and VAT show a significant association, which has also been demonstrated previously.\textsuperscript{2} Given the close proximity between these fat compartments, this association was expected, but whether VAT is the main cause of PFF still remains to be elucidated.

Previously, a positive correlation between BMI and PFF was observed.\textsuperscript{38} Interestingly, in the present study, PFF is higher in subjects with obesity compared with subjects without obesity, yet in the obesity subcohort, no association between PFF and BMI-SDS was established (Table 2) (Fig. 3) despite the relatively wide range of BMI-SDS in this subcohort. This indicates that once obesity is present in adolescence, accumulation of pancreas fat is driven by other factors than total body fat. One relatively small-sized pediatric study has previously reported a difference in PFF between subjects with and without obesity; however, the correlation between PFF and BMI-SDS in regression analysis has not been investigated.\textsuperscript{5} Furthermore, the present study demonstrates that the associations of PFF to other parameters in simple regression models are highly dependent on BMI characteristics of the cohort studied. This could serve as explanation of previously reported conflicting results regarding the association between PFF and estimates of BCF and IR in heterogeneous cohorts.

Multiple techniques can be applied to quantify adipose tissue. With the use of MRI, PFF can accurately be determined and studied in relation to metabolic abnormalities.\textsuperscript{5} Previously, PFF has been measured using computed tomography,\textsuperscript{12} magnetic resonance spectroscopy,\textsuperscript{9,11} and MRI.\textsuperscript{4,5,10,39} Among these, MRI is advantageous because it allows analysis of lipid-specific signals from a large section of the pancreas without using ionizing radiation. The PFF varied between approximately 0% (absent) to 15% in this study. Previously reported amounts of PFF differ; in pediatric subjects, maximum values of 7% to 14.4% have been reported,\textsuperscript{5,10} and adults show similar maximum values.\textsuperscript{8,38–40} One
single study, which used magnetic resonance spectroscopy, reported maximum PFF of more than 50%.

This study is one of the largest examining PFF with MRI in children, but results must be interpreted with some limitations in mind. Subjects were recruited at 2 European sites; however, all examinations were harmonized and carried out according to standard operations procedures. Furthermore, in both subcohorts the range in BMI-SDS was relatively wide (Fig. 3). Because PFF did not correlate with age in either subcohort, puberty was not taken into consideration. Other limitations are that no clamps were conducted, which is the criterion-standard method to assess insulin secretion and resistance and that ethnicity was not controlled for.

We conclude that adolescent subjects with obesity have elevated PFF that is not associated to BCF or IR. Rather, PFF is associated to MetS and VAT. This association study also identified secretion and resistance and that ethnicity was not controlled for.

ACKNOWLEDGMENTS

The authors thank all MRI personnel, Malte Lidström, and Martin Ahlström for their contributions and efforts related to this study.

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